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The Physiology of the Cavitation of Xylem Sap

by

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Dedication

To Peta who got me to Britain and to my parents who encouraged me to stay.

Abbreviations, Symbols and Units

Abbreviations

A	-	Area
DW	-	Dry weight
FW	-	Fresh weight
g_s	-	stomatal conductance
K	-	relative conductivity
K'	-	Permeability of shoots, approximately equal to K.A
l	-	length
P	-	balance pressure
ΔP	-	pressure difference
Q	-	flux
RWC	-	Relative water content
RWC'	-	Apoplastic water fraction
TW	-	Turgid weight
$t_{\frac{1}{2}}$	-	Half time
Vi	-	Volume taken up
Ve	-	Volume expressed

Symbols

η	-	viscosity
Θ	-	contact angle
ρ	-	density
σ	-	surface tension
σ'	-	conductivity
Ψ	-	water potential
Ψ_l	-	leaf water potential
Ψ_p	-	turgor potential
Ψ_p'	-	water potential at incipient plasmolysis
Ψ_s	-	osmotic potential

Ψ_s° - osmotic potential at full turgor

Ψ_x - xylem water potential

Units. Systeme International (SI) units are used in this thesis except for

i) angles, which are given in degrees,

ii) time, which is in units of seconds, minutes and hours.

and iii) As an aid to comprehension volumes are sometimes given in non-SI units of dm^3 (1 dm^3 = 10^{-3} m^3) or litres (1 litre = 10^{-3} m^3).

Where the figure axes include a multiplier this applies to the numerical value entered in the figure and not to the units.

Summary

Four aspects of the physiology of the cavitation of xylem sap were studied.

i) The relationship between cavitation and clicks detected by the acoustic technique.

Further evidence that clicks detected using the acoustic technique were the result of cavitation was obtained. Experiments with Acer showed that the appearance of gas in xylem conduits was associated with clicks. Experiments with Rhododendron showed that the production of clicks was dependent on sap tension and not on cell turgor. On the basis of these experiments and preceding reports in the literature clicks must now be regarded as almost certainly being caused by cavitation.

ii) The effect of cavitation on pressure chamber measurements of water potential.

Experiments showed that cavitation did not cause errors in pressure chamber measurements of water potential of Rhododendron leaves but was a possible cause of error when using samples with a relatively large xylem volume, for example Rhododendron shoots. The pressure chamber could be used to assess sap tensions in cavitating leaves.

Acoustically detected cavitation was found to occur at characteristic sap tensions in eight species of herbs, shrubs and trees. In general cavitation occurred at lower sap tensions in herbs than in trees (0 - 1 MPa and > 1 MPa respectively). Acoustically detectable cavitation was complete by sap tensions of < 3.5 MPa in all species except Fraxinus excelsior.

iii) The factors determining the sap tension at which cavitation occurs.

The pressure required for gas to penetrate wet pit membranes in Rhododendron stems was very close to the sap tensions causing cavitation in Rhododendron leaves (1 - 3.0 MPa). The pressure at which gas penetrated the pit membranes appeared to be determined by the surface tension of the

liquid in the pores of the pit membranes. It is proposed that differences in the size of the pores of the pit membrane are the cause of the differences in sap tension at which cavitation occurs in different species.

iv) The effect of cavitation on the flow of water in the xylem.

The permeability of Rhododendron shoots and stem segments had decreased almost to zero by the sap tensions at which the last clicks were detected by the acoustic technique. Slower equilibration of water potentials amongst leaves on shoots and a decrease in the proportion of the xylem carrying sap as sap tensions increased also indicated that the flow of water in the xylem had been disrupted. However cavitation occurred progressively over a range of sap tensions. Sap tensions of 2 MPa, while reducing stem permeability by 30-50%, failed to affect measurably the supply of water to the leaves of Rhododendron plants. This suggests considerable redundancy in the supply of xylem conduits in this species.

Contents

Chapter 1. Introduction

1.1. General introduction

Our present concepts of the movement of water through plants are based on the cohesion theory proposed by Dixon and Joly (1894). Central to the cohesion theory is the concept that xylem sap in plants must be able to sustain large negative pressures (tensions) without the formation of a gas phase (cavitation). The ability of sap to maintain these tensions depends on the cohesive forces within the liquid and the adhesive forces between the liquid and the walls of the xylem conduits in which it is contained.

The cohesion theory is now generally accepted as being correct in its descriptions of the process of xylem sap transport. However, the ability of xylem sap to withstand the tensions required by the cohesion theory to move water at the rates required by plants has not yet been demonstrated with certainty.

In vitro experiments to determine cohesive limits in water have been subject to many sources of error as well as differing interpretations. Observations of sap movement in plants indicate that xylem conduits quite often cease to conduct sap as they age. High sap tensions which exceed the cohesive limits of the sap and which occur in the later part of the summer are often held responsible for this.

This project was undertaken to determine the limits of sustainable sap tension and to assess the importance of cavitation on the ability of the xylem to transport water. The development of the pressure chamber technique for determination of sap tension (Scholander et al., 1964 ,1965) and of the acoustic technique for detection of cavitation in sap (Milburn and Johnson, 1966) have made this study possible.

1.2. The cohesion theory

Water movement in plants appears to be determined by physical rather than biological processes. For instance, Hales (1738) observed that the amount of water evaporated by a potted plant was dependent on the brightness of the sunlight reaching the plant. Strasburger (1891) showed, by using poisons or heat to kill the living tissues of shoots, that the presence of living cells was not necessary for transpiration to occur.

These and other observations were drawn on in the development of the cohesion theory of sap ascent (Dixon and Joly, 1894; Askenasy, 1895; Dixon, 1914). The cohesion theory describes water movement through plants as being a physical process dependent on inputs of energy from the environment of the plant.

The cohesion theory is based on three tenets. They are:

a) Tension in the xylem sap is created by evaporation of water from the small capillaries of cell walls, mainly in the leaves. This tension is transmitted through the liquid of the xylem sap and causes the movement of water from the soil to the leaves. In consequence, water moves along a gradient of potential energy (sect. 1.3) from the soil where water is present at higher potential.

b) Water in the xylem is stable when under hydrostatic tension whether or not the water contains dissolved gases.

c) The cellular structure of the xylem protects sap columns under tension from the entrance of gas which would destabilise the columns.

The cohesion theory quickly gained wide acceptance and is still considered to be an accurate description of the means by which water moves in plants.

The main arguments against the cohesion theory concern the stability of the sap columns under tension. These arguments are discussed below.

1.3. Water potential and sap tension

Dixon (1914) recognised that water moves through plants from a region of greater potential (in the soil) to one of lower potential (the air). In this respect water movement obeys the second law of thermodynamics.

The chemical potential of water at any point in the soil, plant or air can be described in terms of free energy. In plant water relations the free energy is expressed as water potential, which has units of pressure and is obtained by dividing the free energy of water by its partial molal volume.

Total water potential at any point is the sum of component water potentials of which the osmotic potential (Ψ_s), and the turgor potential (Ψ_p) are usually the most important (Boyer, 1967). The component potentials due to gravity, matrix adsorption and frictive losses are usually omitted (Passioura, 1980; Richter, 1973) as they are small relative to Ψ_p and Ψ_s or are difficult to measure.

Water potential (Ψ) is therefore expressed as (Boyer, 1967)

Eqn. 1. $$\Psi = \Psi_s + \Psi_p$$

As xylem sap Ψ_s is usually near zero (e.g. Boyer, 1967) the xylem Ψ_p is in many cases near to the water potential at that point.

The minimum Ψ attainable is that of the driest part of the environment in which the plant lives, usually the air. The water potential of the air falls quite rapidly with falling humidity, for instance being -94 MPa at 50% relative humidity. The water potential occurring at any point in a plant will lie between that of the soil and the air between which the plant forms a connection (Lange et al., 1976).

Transpiration, soil Ψ and water flow path resistance determine sap Ψ under steady state conditions (Richter, 1973). Sap Ψ_p as low as -16 MPa have been reported for a desert plant and xylem Ψ_p of -4 to -7 MPa are not uncommon (Richter, 1976). Typical woody plants of mesic sites may exhibit xylem sap Ψ_p of between -1 and -3 MPa.

1.4. Sap tension and xylem sap turgor potential

The xylem sap turgor potential, Ψ_p, is the pressure within the sap. When less than the vapour pressure of the sap it can be likened to the tension in a rope with the sap columns of the xylem being the 'rope'. As much of the following discussion deals specifically with xylem sap Ψ_p in its relation to cohesive or adhesive failure in the sap (breaking of the 'rope') xylem Ψ_p, usually a negative quantity, will be replaced by the term 'sap tension', a positive term defined as sap tension $= -[(\Psi_p)_{sap} + \text{atmospheric pressure}]$

$= -[(\Psi_p)_{sap} + 0.101 \text{ MPa}]$

The correction for atmospheric pressure is necessary as the reference state for determination of Ψ is pure water at atmospheric pressure.

1.5. Exclusion of air from xylem conduits

1.5.1. The cell wall

Xylem sap can remain in tension as only the liquid phase exists in the conduit. At all except the very lowest sap tensions any bubble large enough to survive immediate collapse under surface tension will expand and rapidly occupy the entire conduit, rendering it non-conducting. Dixon and Joly (1894) recognised the importance of the xylem structure in preventing such a gas phase entering a conduit.

The walls of plant cells, including those of the xylem conduits, are porous, consisting of variously arranged microfibrils with lignins, pectins and hemicelluloses between them (Chafe, 1970; Berlyn, 1969). The remaining spaces between the microfibrils form capillaries with radii of about 5 nm (Preston, 1952; Nobel, 1970). Capillarity will maintain a meniscus in pores of this size at pressure differentials of up to 29 MPa and so prevent gas penetration following expulsion of water held in the pores by capillarity. As the lowest Ψ recorded in living plants is about -16 MPa, gas entry through cell walls by withdrawal of water from cell wall capillaries is unlikely (Richter, 1976).

Electron microscopy has shown deposits of amorphous materials on cell walls bordering xylem conduits. These deposits would further reduce pore

size and increase the sap tension required to pull meniscii from the cell walls (Schmid and Machado, 1968).

Gas may sometimes enter xylem conduits through leaf abscision scars or wounds. Sap conduction in neighbouring conduits is then threatened by this gas unless the gas is prevented from entering undamaged conduits. Dixon and Joly (1894) recognised the role of the pit membranes in preventing the spread of gas from one conduit to the next. They proposed that sap menisci were retained in the pores of the pit membrane by capillarity.

1.5.2. The pit membrane

Electron micrographs of intervascular pit membranes of Eucalyptus regnans (Cronshaw, 1960) and selected legumes (Schmid and Machado, 1968) fail to reveal pores in the membrane at magnification of $x10^5$. This implies a pore size of 10^{-9} m or less in these membranes. Such a pore would retain a meniscus against pressure differentials of 150 MPa, far in excess of the sap tensions likely in a living plant. Again, amorphous deposits and the 'warty layer' have been found on intervascular pit membranes and will further reduce pore sizes (Cronshaw, 1960; Schmid and Machado, 1968).

Evidence from particulate filtration experiments also suggests very small pit membrane pore sizes in angiosperms. Nemec (1975) found that myelin fibres 300 nm in diameter blocked pit membranes in citrus xylem. Van Allen and Turner (1975) reported variations in the effectiveness of dextrans in causing blockages of vascular pit membranes of several herbaceous species. They interpretated their findings as evidence of species related differences in pit membrane pore sizes. Species with larger pit membrane pores would suffer gas entry at lower sap tensions than those with smaller pores.

The effectiveness of the simple angiosperm pit-membrane in holding menisci has been noted by direct observation of bubbles in soft herbs (see review by Crafts et al., 1949). The multiple-cut experiments of Greenidge (1955b),

Postlethwaite and Rodgers (1958) and Mackay and Weatherly (1973) have all shown that water transport continued in conduits in close proximity to those embolised by the cuts. Pits between the embolised and non-embolised conduits were therefore effective in preventing air entry to non-embolised conduits at pressure differentials of less than 2.6 MPa (Greenidge, 1955a). Gas appeared in the xylem of the deciduous hardwoods of the Greenidge (1955a) study at sap tensions in excess of 2.5 MPa, apparently by cavitation of sap in previously non-embolised conduits. However, Greenidge (1955a) noted that gas did not appear randomly over the xylem but was concentrated in discrete areas which subsequently expanded as sap tensions rose. He offered no explanation as to why gas should appear in localised areas rather than uniformly over the xylem.

Gymnosperms possess a highly specialized type of pit, the bordered pit, in which a dense, apparently non-pervious torus is surrounded by a relatively large porous margo (of pore diameter about 0.1 μm in Abies (Petty and Puritch, 1970) . The pores of the margo are therefore too large to prevent gas entry at sap tensions greater than 1 to 1.5 MPa. Before the menisci are dislodged from the membrane pores the torus is moved sufficiently to block the pit annulus and so prevent gas entry (Gregory and Petty, 1973; Chapman et al., 1977). However, Bailey (1916) was able to blow air through blocks of conifer wood by using pressures of 0.3 - 1.4 MPa. This result may be evidence of some faulty toral sealing of the bordered pit.

1.6. Gas phase formation in liquids

A gas phase may arise within sap which is under tension. The gas phase will expand to occupy the entire conduit if the pressure of the xylem sap is much less than that of the vapour phase of the gas. This will usually be at sap pressures of 0.01 MPa or less. Gas phase formation in this manner is termed cavitation.

Cavitation in marine and hydraulic systems, such as turbines and

propellers, is a common phenomenon and occurs at fluid tensions of 1 MPa or less (Hammitt, 1980).

Cavitation is also responsible for causing the bends in divers. The bends occur when nitrogen gas, dissolved in body fluids when diving, comes out of solution and forms bubbles when pressure is released upon surfacing. The bends may occur when nitrogen has been forced into solution at pressures of as little as 0.2 MPa above atmospheric (Walder *et al.*, 1968).

Cavitation in engineering and animal systems is therefore known to occur at fluid tensions small by comparison with those found in plant xylem sap. How do plants escape cavitation of their sap at tensions greatly in excess of those at which cavitation occurs in other systems?

Cavitation may occur as a result of bubble formation *de novo* or from the expansion of a bubble from a small, pre-existing nucleus.

1.6.1. Bubble formation *de novo*

Bubbles forming *de novo* may be the result of failure either of cohesive forces between the molecules of the sap or of the adhesive forces of the bonds between the molecules of the sap and those of the xylem conduit in which the sap is contained.

Cohesive failure is an highly unlikely occurrence at the sap tensions likely in plants (Greenidge, 1952; Oertli, 1971) as water has a very high capacity for hydrogen bond formation (Nobel, 1970). Bubbles formed *de novo* at sap tensions less than about 10 MPa are very likely to collapse immediately under pressures developed by surface tension (Oertli, 1971).

Experimental determinations of cohesive limits in water are difficult because of the difficulty of removing gas nuclei from the apparatus. Hayward (1964), using a modified Berthelot tube, obtained maximum liquid tensions of 4 MPa. However, Briggs (1955) used a centrifugal method to obtain liquid tensions of 20 to 30 MPa at temperatures found in biological systems. The difference between the results obtained by Hayward and by Briggs was probably

due to incomplete removal of nucleation sites for bubble formation in the Berthelot tubes used by Hayward. As sap tensions in plants from mesic sites may be 2 to 4 MPa and those from extreme xeric sites may be 16 MPa the results of Briggs (1955) would indicate that cohesive failure is unlikely to be the cause of cavitation in plants.

As cell walls are formed fully wetted by precipitation from solution it is extremely unlikely that xylem conduit walls will include nuclei from which bubbles may expand. Also, the major constituents of cell walls, celluloses and lignin, have structures with a high capacity for hydrogen bonding with water so that adhesive failure is unlikely at low sap tensions (Greenidge, 1952). However, adhesive failure may occur at low sap tensions by shearing as sap moves over the solid cell wall surfaces (Hayward, 1967).

1.6.2. Bubble expansion from pre-existing nuclei

A small bubble may be saved from immediate collapse due to surface tension if it is protected in a crevice (Harvey et al., 1944). The bubble is then stable and may survive until sap tension exceeds the surface tension restraining the bubble in the crevice at which time the bubble will expand and cavitate the conduit. Even short periods of high sap tension, e.g. caused by sonic vibrations, may act cumulatively to expand a bubble sufficiently to overcome surface tension and continue its expansion (Harvey et al., 1944). This is the basis of ultrasonic degassing of water.

As the formation of small bubbles is more likely than the formation of large ones (Oertli, 1971) the presence of suitable crevices may make cavitation more likely. Bubbles may also be carried into the xylem in crevices in small particles contaminating the xylem sap. Such particles are unlikely to enter with the transpiration stream because of the filtering action of cell walls, and particularly the endodermis, of the roots, and subsequently of the pit membranes. Nuclei may however enter as contaminants in sap retreating from

damaged conduits, for instance as a result of wounding. These particles will only be effective in causing cavitation if they are small enough to pass through the pit membranes and into undamaged conduits.

1.7. Observations of cavitation in xylem

Crafts et al. (1949) review experiments in which emboli were caused to form in the xylem of water-stressed plants by jarring the stems. Observations of gas in the xylem of trees were made by Haines (1935). The number of gas-filled conduits apparently increased with increasing sap tension. His results may have included conduits which had cavitated while being prepared for viewing although he tested for this by injections of eosin dye. Uptake of dye indicated that the xylem conduit contained either sap under tension or gas at reduced pressure (and therefore present as the result of recent cavitation such as might occur during preparations for viewing). No uptake of the dye indicated that the conduit was full of air or water at atmospheric pressure and therefore had presumably cavitated some time before. A similar line of reasoning was used by Milburn and McLellan (unpub) who used different coloured particulate and non-particulate dyes to identify water and gas-filled conduits in Ricinus.

Cary et al. (1968) and West and Gaff (1971) cited anomalies encountered while making plant-water-potential measurements as evidence that a gas phase appeared in the xylem conduits at about 0.3, 2.7 and 2.0 MPa of sap tension in tomato, corn and apple respectively.

The acoustic method (Milburn and Johnson, 1966) is claimed to detect the vibrations produced when strain in the elastic walls of xylem conduits containing sap under tension is released by the formation of a gas phase. This occurs at sap tensions of about 1.0 MPa in leaves of Plantago (Milburn and McLaughlin, 1974) and 0.7 MPa in Ricinus (Milburn, 1973b).

The experiments described above generally involved some degree of damage

to the xylem prior to observations being made. As a result air was admitted to at least some of the xylem conduits. The possibility that the gas phase releasing sap tension enters the conduit from outside, rather than arising within the conduit, therefore cannot be discounted.

1.8. The effect of cavitation on sap transport

The movement of sap in the xylem in response to a pressure gradient is usually described using some form of the van den Honert (1948) electrical analogue. This analogy likens the flow of sap to an electrical current, increasing with increasing pressure gradient (voltage) and decreasing with increasing resistance to flow.

Heine (1971) recognises two terms describing the flow of sap or water through the xylem. They are relative conductivity, K, and conductivity, σ.

Relative conductivity makes a minimum number of assumptions about the structure and function of the xylem segment in question. The xylem is modelled as a heterogeneous porous matrix and flow through it, as expressed by the relative conductivity, K, is derived from measurements of sap flux resulting from an applied pressure gradient applied to the segment.

Conductivity, σ, assumes that flow in xylem can be described by the same equations as flow in pipes. Measurement of σ requires a knowledge of xylem dimensions and, for a complete description of flow, the flux in each conduit.

1.8.1. Relative conductivity, K

Heine (1971) defines relative conductivity, K, as

Eqn. 2. $K = \frac{Q.l.\eta}{\Delta P.A}$ in which Q = volume of fluid passing a plane at right angles to the direction of flow per unit time.

l = the length of xylem over which the flux, Q, occurs.

η = viscosity of the fluid.

ΔP = the pressure difference driving flow.

A = the cross-sectional area of the xylem.

If this model adequately describes flow in the xylem, the fluid flux Q will be linearly proportional to A, ΔP and K but inversely proportional to l.

Cavitation will result in fewer conduits in the xylem cross-section being available for sap transport. As the total xylem area remains the same as before cavitation, the relative conductivity of the xylem will decrease although the ability of remaining, non-cavitated conduits to conduct may also remain the same.

Measurement of K therefore provides a quantitative measure of the ability of the xylem sample, as a whole, to transport water or sap. At the same time a minimum of knowledge of the structure of the xylem in the sample is required so that K can be calculated from simply made measurements of Q, A, l and ΔP.

Relative conductivity and permeability

Permeability is sometimes used as a synonym for relative conductivity (Booker, 1977).

1.8.2. Conductivity, σ

The quantity σ is defined by Heine (1971) as

Eqn. 3. $\sigma = \frac{v.l}{\Delta P}$ in which l and P are as for the calculation of K.

v = the velocity of the sap moving in the xylem.

This expression for σ applies only to xylem made up of one or more conduits of the same dimensions as, by application of the Poiseuille equation for flow in pipes, v is proportional to the fourth power of the pipe radius. Therefore conduits of different radii will have very different σ values.

Conductivity can also be defined (Heine, 1971) in a form which describes fluid movement in a manner similar to that for K but substituting the area of vessel luminae, a, for the total xylem area A. The equation has the form

Eqn. 4. $\sigma = \frac{Q.l}{\Delta P.a}$

Again this equation must be modified for variation in conduit radii.

By measuring all conduits in each class of lumen dimension and by calculation of σ before and after cavitation it may be possible to learn more of the effects of cavitation on sap transport in conduits of different dimensions.

In practice it is tedious to measure all dimensions of all conduits in a xylem section and to sort these into appropriate classes, and impossible to establish any other than a maximum velocity for the sap. This maximal velocity is very likely to be that in the conduits of greatest radius.

The value of measurements of σ may be further reduced for studies of the effect of cavitation on flow as there is evidence that, perhaps because of the porosity or sculpturing of xylem conduit walls, sap flux in a single conduit may not be simply related to the pressure driving flow (Giordano et al, 1978; Jeje and Zimmermann, 1979).

1.9. Removal of gas from embolised conduits

Embolism of the xylem conduits may harm a plant by restricting the ability of the xylem to supply sufficient water for the plant's requirements. This effect will be greatest when plant water demand is high. However, transient water deficits are common in plants and may not result in permanent damage. The midday minimum in Ψ is a well-known example of such a transient deficit (e.g. Hinckley et al., 1975). Cavitation of xylem sap will tend to exaggerate such phenomena but, unless flow is stopped completely or seriously impaired for a long period, its effect will not be great. If cavitation occurs to such an extent that plant Ψ cannot be maintained adequately restoration of conductive capacity by refilling embolised conduits is necessary to avoid interruption to the plant's functioning.

Bubbles in the xylem conduits will dissolve if the pressure in the bubble is less than that exerted by hydrostatic pressure and surface tension together.

This will not occur if sap tension is high (Dickson and Blackman, 1938) and will depend on the radius of the conduit containing the bubble and the composition of the sap. If positive sap pressures occur, for instance through root pressure, conditions may be suitable to force bubbles into solution. Gravitational potentials may also be significant in taller plants in this respect.

Direct visual observations of solvation of bubbles in the xylem of Impatiens when water is made freely available have been made (Dickson and Blackman, 1938). Crafts (1939) made similar observations of bubble collapse in the xylem conduits of intact Ribes inerme when water stress was relieved by rewatering. Interestingly bubble collapse was most rapid in the narrower conduits, possible confirmation that surface-tension-derived pressure on the bubble is important in removal of emboli as this pressure is greatest in the smaller conduits (Nobel, 1970).

1.10. Sap tension and xylem anatomy

Dixon (1914) recognised that the xylem is particularly suited to resist collapse under the differential pressures occurring when the sap it contains is under tension. The xylem is divided into a large number of small conduits which often have heavy secondary thickening as spirals, rings or reticulate networks on the inner surface.

The xylem of plants growing in xeric or mesic environment was compared by Starr (1912). Specimens from a xeric environment generally had vessel elements which were narrower, shorter and with thicker walls than those of examples of the same species taken from a more mesic site. After examining the xylem of a large number of species from different environments Carlquist (1975) considered that the shorter, narrower and thicker-walled vessel elements of plants from arid climes compared to those from wetter areas to be an adaptation conferring tolerance of higher sap tensions on the desert plants. Similarly he considered the shorter and narrower vessel elements of tree crowns

compared to those of the more basal parts to reflect the general increase in sap tensions occurring from roots to leaves.

The apparent relationship between sap tension and vessel anatomy may be coincidental though. Water-stress reduces the amount of photosynthate available for wall development (Doley, 1970; Smith, 1976) and turgor for cell expansion (Jones and Turner, 1980; Sands and Correll, 1976). These reductions can lead to shorter and narrower vessel element being formed.

Structural differences within plants also serve to minimise sap tensions in the trunk and major branches during short periods of low leaf Ψ. The connection resistance of the petiole (e.g. Begg and Turner, 1970; Landsberg et al., 1976) which imposes a high sap tension gradient over a short distance is probably due to a zone of reduced vessel diameters in the petiole (Larson and Isebrands, 1978). A high petiolar resistance may act in series with laminal resistances to water flow to effect a rapid reduction in guard cell water potential when atmospheric humidity falls. This may result in a more sensitive stomatal response to changes in evaporative demand (Tyree and Yianoulis, 1980).

Zones of constricted vessel diameter at branch points and lower xylem permeabilities in the lower and lateral branches also serve to protect the xylem of trunk and root from transitory high sap tensions and to determine the distribution of water with the crown (Zimmermann, 1978).

Considerable differences in wood anatomy occur between the gymnosperms and the angiosperms, particularly the large porous deciduous trees, with which they sometimes co-exist. These differences are related to the ecological strategies for water transport in each of these groups. The deciduous trees supply foliar water requirements through a relatively small number of long vessels of large diameter (Zimmermam and Jeje, 1981). The large diameters and long, uninterrupted lumina of these vessels allow high sap flow rates to be achieved with low pressure gradients. However, the smaller number of conduits leaves these trees susceptible to water stress if, by freezing,

disease or cavitation under high sap tension, these vessels are embolised. The shorter, narrower tracheids of conifers require greater pressure gradients to achieve the same rates of flow as in the stems of the deciduous trees but their greater number minimises the effect of embolism of some of them (Zimmermann, 1974). As a large proportion of the conducting vessels of the deciduous trees are embolised by high sap tensions in summer (e.g. Greenidge, 1955a) or by freezing in winter (e.g. O'Malley, 1979) these trees must replace their conducting vessels each spring at considerable metabolic cost (Zimmermann and Brown, 1974). By contrast the xylem of conifers may remain active for several years, minimising the metabolic cost of maintaining sap flow as only part of the conductive capacity need be replaced each year. However, this saving is achieved at the cost of higher resistances to sap flow and lower crown water potentials or reduced rates of water transport.

1.11. The aims of the project

Reports of xylem sap cavitation occur with some frequency in the literature, often indirectly as an aside to other work. The relation between sap tension, xylem anatomy, sap cavitation and xylem sap flow has had little study.

In view of the critical importance of the stability of xylem sap when under tension to the cohesion theory and the ability of the plant to transport water, the following research was undertaken to investigate the occurrence of xylem sap cavitation and its effect on water transport in the plant.

This work is made possible by the development of the pressure chamber for the rapid measurement of sap tension (Scholander et al., 1964 ,1965) and of the acoustic technique for the monitoring of cavitation in nearly intact xylem (Milburn and Johnson, 1966).

These techniques have been used to seek answers to the following general questions.

1) Are clicks detected by the acoustic technique actually due to cavitation in the xylem? If so, at what sap tensions do they occur in different species?
2) To what extent does cavitation affect the ability of the xylem to conduct sap? Is the effect reversible upon supply of water at high water potential?
3) Is the use of the pressure chamber for measuring sap tension affected by cavitation? The pressure chamber technique is based on the assumption that xylem sap is distributed similarly in a leaf or twig held at the balance pressure in the pressure chamber and in the twig attached to the plant. This may not be the case in cavitated material.

As mentioned earlier (section 1.4) a positive quantity, sap tension, which is equal to the negative of xylem sap turgor will be used to describe the stress to which the xylem sap is being subjected. The pressure chamber provides a method of determining sap tension directly as, at the balance pressure, chamber gas pressure is equal to $-\Psi_p$ of the sap before sampling (section 2.1) (Scholander, 1964). Balance pressure (P) is therefore equal to $-\Psi_p$ of the xylem sap and equal to sap tension plus atmospheric pressure.

The acoustic technique is used to determine both the sap tensions at which cavitation occurs and also to assess the extent to which embolism has progressed or been reversed in samples used in other experiments.

Chapter 2. Materials and Methods

2.1. Plant material

Leaves, twigs or whole plants to be used for experiments were collected from the field or grown in a glasshouse. The groups were designated as field (F) or glasshouse (G) collections as appropriate.

Field collections: Samples were collected from the grounds of the Garscube Estate, Bearsden, Glasgow. Samples from trees and shrubs were taken 0-3 metres above the ground and, as far as possible, from branches fully exposed to the sun on the southern side of the plant.

Species from which field collections were made were *Acer pseudoplatanus* L., *Alnus glutinosa* L., *Fraxinus excelsior* L., *Larix decidua* x *kaempferi* Mill., *Plantago major* L. and *Rhododendron ponticum* L.

Glasshouse collections: Plants were grown in 1:1:1 peat/soil/sand mixture in free draining plastic pots or root bags of appropriate size and kept well supplied with water.

Liquid fertilizer (Liquid Garden 'Plus', ICI, Farnham, UK) was applied at intervals.

With the exception of the *Eucalyptus globulus* Labill (L'Hérit.) specimens supplementary lighting was applied during the winter months. Mercury vapour lamps (400W, 'KOLORLUX' MBFR/U, Atlas, UK) one metre above the glasshouse bench were used.

Lycopersicum esculentum (cv. Moneymaker) Mill., *Phaseolus vulgaris* (cv. Canadian Wonder) L., *Plantago major* L. and *Ricinus communis* L. were grown from seed. *Acer pseudoplatanus* seedlings were bought from the Ben Reid Nurseries Ltd., Aberdeen, UK and were 5-6 years old when used. *Rhododendron ponticum* shrubs in 100 mm root bags were bought from Findlay-Clark Nurseries, in February 1982 and used between June and July of the same year. Samples of

Eucalyptus globulus were taken from a 3m plant rooted in the floor of the glasshouse. Samples were also collected from self-propagated Zebrina pumulus L. vines growing on the floor of the glasshouse..

2.1.1. Collection and hydration of samples

Samples were collected in the late afternoon when water potentials would be expected to be recovering from the midday minimum. Leaves or twigs were cut under water from the plant and recut to remove embolii. Leaves and twigs were brought to full turgor ('hydrated') by standing overnight with the cut surface in distilled water in an insulated box lined with wet paper towel. Twigs too big to fit in the insulated box were enclosed in polyethylene bags secured below the lowest leaves and the cut end of the stem supplied with distilled water. Material was usually used within one day, and never kept for more than two days before use.

Rehydration of material after periods of stress was by the same procedure as described for the initial hydration of samples.

2.2. The Acoustic technique for the detection of cavitation

Cavitation was monitored as it occurred by the acoustic technique (Milburn and Johnson, 1966; Milburn, 1973a). Vibrations, attributed to the sudden release of wall tension due to the formation of a gas phase in a xylem vessel and its expansion, are detected by a sensitive transducer coupled to the xylem through a metal needle. If amplified and played through a loud speaker each cavitation event produces a 'click' sound. Hereafter acoustically detected cavitation events will be called 'clicks' for convenience.

The general procedure involved in conducting an experiment relating cavitation to sap tension was to monitor click production using the acoustic detector, and at intervals to note leaf weight (and from this and leaf dry weight to obtain RWC). Clicks were then related to the sap tension at which they occurred through a 'calibration' of sap tension against RWC (section 3.1)

compiled using leaves similar to those used for acoustic experiments.

The nature of the equipment available dictated that two methods be used to measure weight changes during the study although treatment of the data so compiled was the same.

2.2.1. Initial experiments

Initial experiments were conducted using equipment very similar to that of Milburn (1973a,b) and Milburn and McLaughlin (1974). This consisted of a magnetic microphone as the detecting transducer which was coupled to the xylem by a steel or copper needle soldered to the armature. The signal produced by the transducer was amplified by a battery-powered circuit which included an adjustable band pass filter and variable gain. The amplified signal was prepared for recording by a discriminator consisting of a trigger circuit which, when tripped, lit an incandescent bulb. A photo-electric cell mounted above the bulb produced an EMF which was recorded by a chart recorder.

The magnetic transducer and preamplifier housed with it was heavy (about 45 gms) and the shielded multicore cable connecting the preamplifier to the main circuitry imposed a considerable resistance to flexing. As a result, it was impossible to record changes in leaf weight of less than about 0.1 g when weighing the leaf and transducer assembly together. Therefore the leaf had to be periodically removed from the transducer, weighed to 10^{-3}g on a sensitive balance and replaced on the detector if small leaf weight changes were to be measured.

This approach had several serious deficiencies. Clicks could not be recorded while the leaf was being weighed, hampering attempts at quantification of click production. Removing the leaf from the acoustic cabinet usually meant a sharp reduction in transpiration so that a smooth decrease in water content with time was probably not achieved. To minimize disruption to the rate of water loss and to the xylem by repeated insertion of the coupling needle, the leaf could only be weighed infrequently, in practice at intervals

of about fifteen minutes. Consequently, when calculating the sap tension at which clicks occurred, errors involved in the production of a calibration of sap tension against RWC were compounded by inaccurate knowledge of RWC of the leaf on the probe at a given time.

The electronic equipment itself had serious deficiencies. Being battery powered its gain did not remain constant as the batteries ran down. Consequently, sensitivity declined gradually until the batteries were replaced, itself an all too frequent occurrence. Although not seriously affecting individual experiments this decline in sensitivity made comparisons of click loudness and number between experiments impossible. The circuitry was also prone to drift if used for long periods and would eventually require resetting of the trimming potentiometers. The circuitry was especially unstable at the high gains required when investigating cavitation in soft herbs.

Experiments conducted with this apparatus included the compilation of cavitation profiles for Rhododendron ponticum, Eucalyptus globulus, Fraxinus excelsior and incomplete experiments using Pelargonium zonale and Zebrina pumulus made in 1979 and the early part of 1980.

2.2.2. An improved acoustic apparatus

A new apparatus was required which would satisfy the following requirements: a) Have a detector (transducer and coupler) which was light enough and connected to the amplifier stages by sufficiently flexible wires to enable leaf weight changes of 10^{-3}g or less to be followed when the leaf and detector were suspended from a balance during experiments.

b) Have a sufficient range of gain and discriminator settings to detect clicks in soft species and to give maximum click frequencies less than that at which clicks become difficult to separate on the recorded charts.

c) Have a facility for suppressing noise at low discriminator threshold settings.

d) Preferably have a multiple channel design so that more than one

experiment may be run at a time.

The arrangement which was arrived at satisfies all these requirements. Its design is sketched in figure 1 and the complete circuit described in appendix 1.

The heavy magnetic transducer of the Milburn apparatus has been replaced by a much lighter (about 5g) detector made by glueing the coupling needle to the stylus of a cheap ceramic record player pick-up cartridge (an X5H Stereo cartridge (BSR, Warley, UK) was used). The preamplifier stage has been moved to a metal case separate from the detector and connected to it by 40 gauge (.122 mm) enamelled copper wire. The light detector supported from the balance arm is now sufficiently free to follow changes of 10^{-3}g in leaf weight. A shielded multiflex cable leads from the preamplifier box to the main amplifier in which the signal is led through the filter circuits and further amplified before entering the discriminator dircuits. Any signals appearing simultaneously on both channels (see below) were cancelled against each other by an anticoincidence circuit. Output connections before the anticoincidence circuit enabled the reference and primary detector channels to be run as separate acoustic detection devices, each with its own discriminator.

The arrangement was found to constitute an aerial for electromagnetic transmissions. Consequently at high sensitivity it could only be used inside a Faraday cage. Fortunately the metal walls of the soundproofed box in which it was housed formed such a cage. The incandescent lamp used to speed transpiration during experiments was shielded by a steel gauze, and metal parts of the balance and the preamplifier box were also earthed through the Faraday cage.

The acoustic detectors must be isolated from external vibrations, not only from the air as sound or draughts, but also through the bench. To this end the acoustic detectors were housed in a double walled metal box with 50 mm of felt soundproofing between the walls. The box floor and door were of wood overlaid with cotton wool. The box stood on layered piles of fibre matting and soft foam plastic at each corner. The whole was supported on its own table in a quiet part of the laboratory.

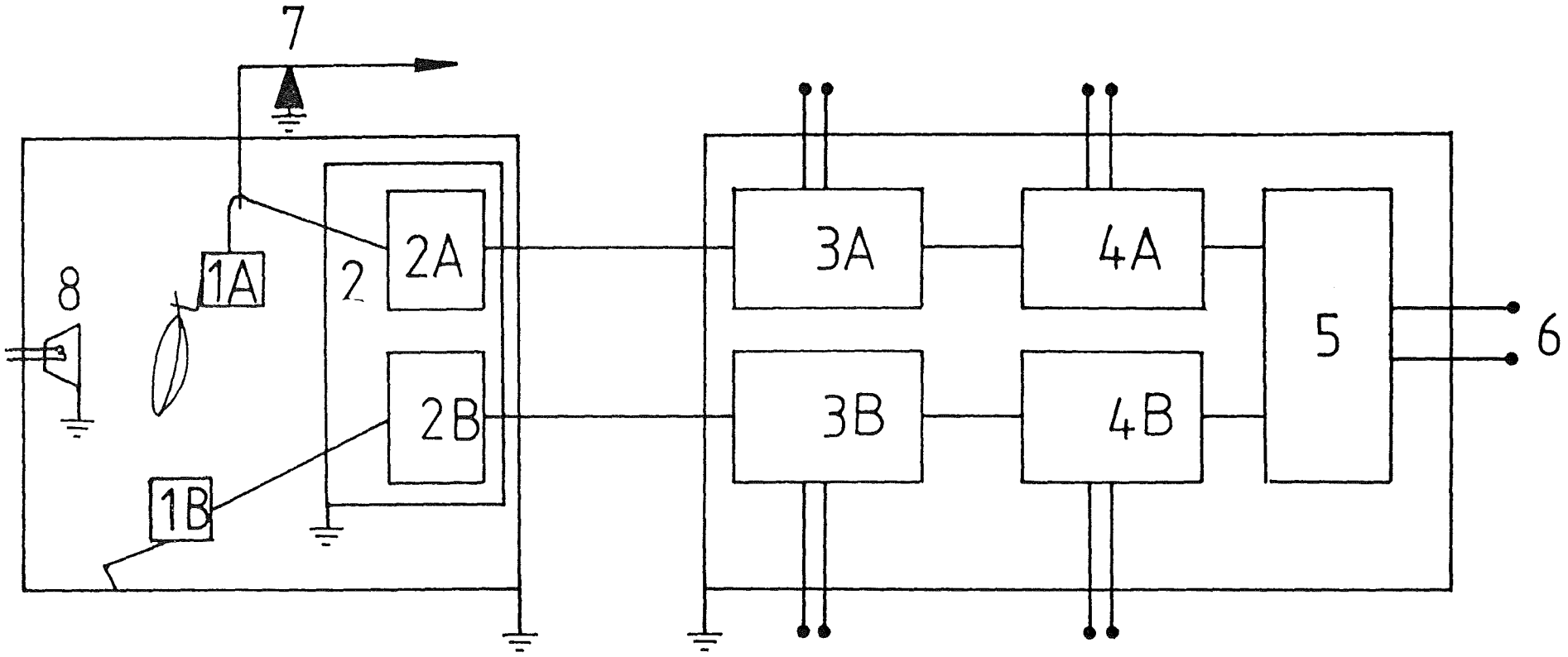


Figure 1. Schematic diagram of the acoustic detector. A leaf was mounted on the transducer (1A) suspended from a balance (7) and transpiration accelerated by an incandescent lamp (8). Transducer output was amplified in preamplifier (x3600) (2A) and main amplifier (x8) (3A) stages and 'clicks' selected by a threshold discriminator (4A). Outputs from the active channel (A) and an identical 'control' channel (B) were cancelled against each other in an anti-coincidence circuit (5) before 'clicks' were automatically recorded (6).

The detector was suspended by two lengths of stiff light wire hooked together and passing through a 10mm hole in the ceiling of the cabinet to the suspension point of a gravimetric balance weighing to 10^{-4}g.

The electronics, except for the detectors, preamplifiers and the chart recorders, were on a separate bench to minimize vibration and reduce electromagnetic interference. The second channel of the amplifiers was used as a reference to detect background noises and cancel them from the output of the detector carrying the leaf. A detector similar to the first was positioned with its needle touching the wooden floor of the box for this purpose.

Stimulation of transpiration

Transpiration by leaves in the box was very slow unless speeded by use of an incandescent lamp. Tungsten filament lamps of 25 to 150 Watt output were used as appropriate to give experimental durations of 1-2 hours. These lamps gave a radiation load at the leaf surface of 30-150 W.m^{-2} (measured by Kipp Solarimeter, Kipp and Zonen Ltd., Delft, Holland).

2.2.3. Conducting an acoustic experiment

Before conducting detailed experiments from which limiting sap tensions were to be derived a number of brief experiments were run using similar material. These trials were necessary for (1) the setting of discriminator thresholds such that the maximum recorded click frequency was at about 40-50 clicks per minute (the maximum frequency at which clicks could be separated on the chart record) and so obtain the greatest resolution of clicks from the background noise. (2) to change the lamp if necessary so that experiments would take one or two hours to complete. This order of magnitude in the duration of the experiments was necessary to obtain sufficient resolution in changes of RWC during the experiment to be able to use RWC as the basis from which to determine sap tensions in the leaf on the acoustic probe. (3) to establish the highest sap tension to which the relation of balance pressure (P) to RWC need be

determined.

Fully detailed acoustic experiments were conducted as follows: An hydrated leaf was weighed to obtain its turgid weight. The leaf was then quickly mounted on the acoustic detector by inserting the detector needle into the xylem of the petiole or stem. Leaves were usually hung from the needle of the acoustic detector as shown in figure 1. Heavier shoots which might damage the detector transducers were hung from the arm of the balance and the detector attached unweighted to the stem.

The lamp, amplifier and chart recorder were switched on and noise levels, particularly due to 50 Hz mains hum, checked to be within tolerable limits. Detector sensitivity was checked by lightly brushing the detector needle with a hair (Milburn and Johnson, 1966).

The weight of the leaf was noted at intervals and the experiment stopped when click frequency had fallen to a frequency small compared to that at the frequency maximum earlier in the experiment. Leaf weight was noted more frequently when the change in weight was rapid (at the beginning of the experiment) than when the rate of loss had declined towards the end of the experiment. Concurrence of clicks with events being recorded by the chart recorder was checked at intervals during the experiment by using a pair of headphones.

Experiments conducted with deeply dissected (e.g. Fraxinus and Lycopersicum) or soft (e.g. Plantago, Ricinus) leaves suffered particularly from rubbing noises. In these cases the laminal parts were held separate by holding them against a light wire frame by using small pieces of putty.

After the experiment the leaf was dried for two days at 363K and then weighed. Clicks were read from the recorded traces and summed over intervals, usually of four minutes. Manual counting of clicks from the recorder charts, although tedious, was preferred to full automatic recording as brief periods of noise, for example due to electrical faults or unusual activity in the laboratory, could be distinguished from clicks on the recorder chart and left out of the summation.

The records of click frequency, leaf weight and time were put onto computer file for later calculation of limiting sap tensions.

2.2.4. Artifacts in acoustic experiments

The acoustic technique is subject to several types of error which may influence the interpretation of experiments and should therefore be borne in mind when interpreting the chart record of an experiment.

Spurious noises arising either inside or outside the sample on the acoustic detector are the major errors to which the acoustic technique is subject.

External noises are the result of vibrations of sufficient magnitude to trigger the active channel discriminator but not that of the reference channel. Airborne noises (e.g. aircraft overhead, whistling or shouting) were the worst sources of this type of noise. Floor-borne vibrations (people walking or working nearby) were usually successfully cancelled out by the anticoincidence circuit.

The second class of external noises was electrical in origin. Mains frequency hum was a serious problem, particularly when low discriminator thresholds were used, but could be suppressed by the shielding and earthing procedures detailed above and by use of the filters built into the amplifiers.

Noises arising within the specimen include tissue noises (Milburn and Johnson, 1966) and noises due to the rubbing against each other of parts of the sample on the acoustic detector.

Tissue noises are usually quiet by comparison to clicks and could be successfully avoided by suitable setting of discriminator thresholds. Tissue noises could not be avoided this way when leaves of soft herbs (Lycopersicum, Pelargonium and Phaseolus) were used as clicks are also very quiet in these species. Manual monitoring of clicks was often necessary with these species. Tissue noises sound quite different to clicks (Milburn and Johnson, 1966) and can be successfully separated from clicks in this way.

Rubbing noises were quite as loud as clicks and sound very similar. Manual recording of clicks and high discriminator thresholds will not therefore separate noises due to the rubbing of leaf parts from clicks. Selective removal of leaves of leaflets was successful in reducing these noises to low levels in Rhododendron shoots and the trifoliate leaves of Phaseolus. In the case of Larix removal of needles could not be used to prevent rubbing noises without removing almost the entire transpiring surface. Rubbing noises in Fraxinus, Lycopersicum and Plantago could be prevented by holding each leaflet or the edges of the lamina against a light wire frame as described above. This method of preventing rubbing also prevents damage to the xylem occurring when parts are excised.

Failure to obtain good contact between the detector needle and the sample xylem was a problem with species in which the xylem occurs in many small vascular strands. Zebrina is one such and the cereals in general are another group for which a diffuse xylem is a problem. All that can be done is to take care to position the needle in as many vascular strands as possible and use the greatest detector sensitivity possible.

The drying of areas around the site of the detector needle insertion might result in a change in the efficiency of the transfer of xylem-borne vibrations to the detector needle, effectively altering the amplitude required by a click to pass the discriminator. Attempts to detect clicks by holding a detector against the outside of the stem or petiole, and thereby avoiding damage to the xylem and epidermis, were unsuccessful. In almost all cases damping of vibrations by soft tissues between the xylem and the detector prevented detection of clicks. Removing these soft tissues to place the detector directly against, but not into the xylem, caused much more rapid drying of tissues around the site at which the detector was mounted than elsewhere in the leaf. Because of this uneven drying it was impossible to determine the sap tension within the sample.

Considering the problems of gaining adequate contact of the detector with

xylem, and of drying around the detector mounting site, a needle attached to the detector was thought the best method available for coupling the transducer to the xylem.

2.3. Measurement of Water Potentials

Routine measurements of plant water potentials were made using the pressure chamber (Scholander et al.,1964 ,1965) and the dewpoint thermocouple psychrometer (TCP) (Campbell et al., 1973). Freezing point osmometry was used in the early stages of the project for measuring solute concentrations, but was superceded by the thermocouple psychrometers when they became available.

2.3.1. Thermocouple Psychrometry

Water potentials (Ψ) were measured using a Wescor Dewpoint Microvoltmeter (Wescor Inc., Logan, Utah) (Campbell et al., 1973) coupled either to a Wescor C-51 sample chamber or sample chambers built for the project to a design supplied by Dr. W.J. Davies of the Department of Biological Sciences, The University of Lancaster. The construction, calibration and operation of the chambers are detailed in appendix 1.

2.3.1.a. Leaf water potentials (Ψ_l). Leaves were prepared for sampling by wiping them with a wet towel to remove surface contaminants and then allowed to dry for at least 20 minutes before sampling. A paper punch was used to cut 6mm diameter leaf discs from halfway along the lamina and midway between the midrib and edge of the lamina. Leaf discs were quickly sealed into the sample chambers and equilibrated for 18-20 hours at 293K. Changes in Ψ_l as a result of metabolic activity in the leaf discs, or slow leaks from the chambers, may have occurred over these long equilibration times. However, these possible errors were considered small and preferable to unknown changes in Ψ_l which might result from abrasion of the leaf surface to achieve more rapid equilibration (Talbot et al., 1975).

2.3.1.b. Leaf solute potential (Ψ_s). Leaf solute potentials (Ψ_s) were measured after destruction of cell membranes, and hence turgor, by freezing. Leaf discs were frozen in liquid nitrogen, warmed briefly between thumb and forefinger and replaced in the sample chambers to equilibrate before measurement of Ψ_s. Equilibration after freezing was obtained within two hours, approximately one tenth the time required when using living leaf discs.

Time courses for equilibration of leaf discs in the psychrometer sample chambers are shown in figure 2.

Cell turgor (Ψ_p) was calculated as the difference between leaf and solute water potentials, i.e.

$$\Psi_p = \Psi_l - \Psi_s$$ (e.g. Bennett et al., 1981)

2.3.1.c. Xylem sap osmotic potential. Xylem sap was expressed from leaves or shoots in the pressure chamber by applying an overpressure of about 0.5 MPa. Expressed sap was absorbed onto 6mm discs of Whatman number 1 filter paper. The discs were enclosed in small plastic film envelopes to minimize evaporation during collection.

Erroneously low sap Ψ_s was measured if small quantities of sap were absorbed onto the paper discs. This problem has been attributed to adsorption of sap water onto the components of the paper, resulting in a concentration of solutes in the remaining unbound sap (Markhart et al., 1981). This artifact was avoided by collecting a sufficient volume of sap so that sap could be squeezed from the paper disc by light finger pressure.

2.3.2. Freezing point osmometry and refractometry of pith sap

Osmotic potentials of sap samples were estimated from measurement of freezing point depression and refractive index of aliquots of expressed sap. Assumptions were then made as to the solutes present in the sap and measurements of osmolality used to derive Ψ_s.

Freezing point depression was measured using a Knauer semi-micro osmometer (Herbert Knauer and Co., Berlin, West Germany) calibrated with

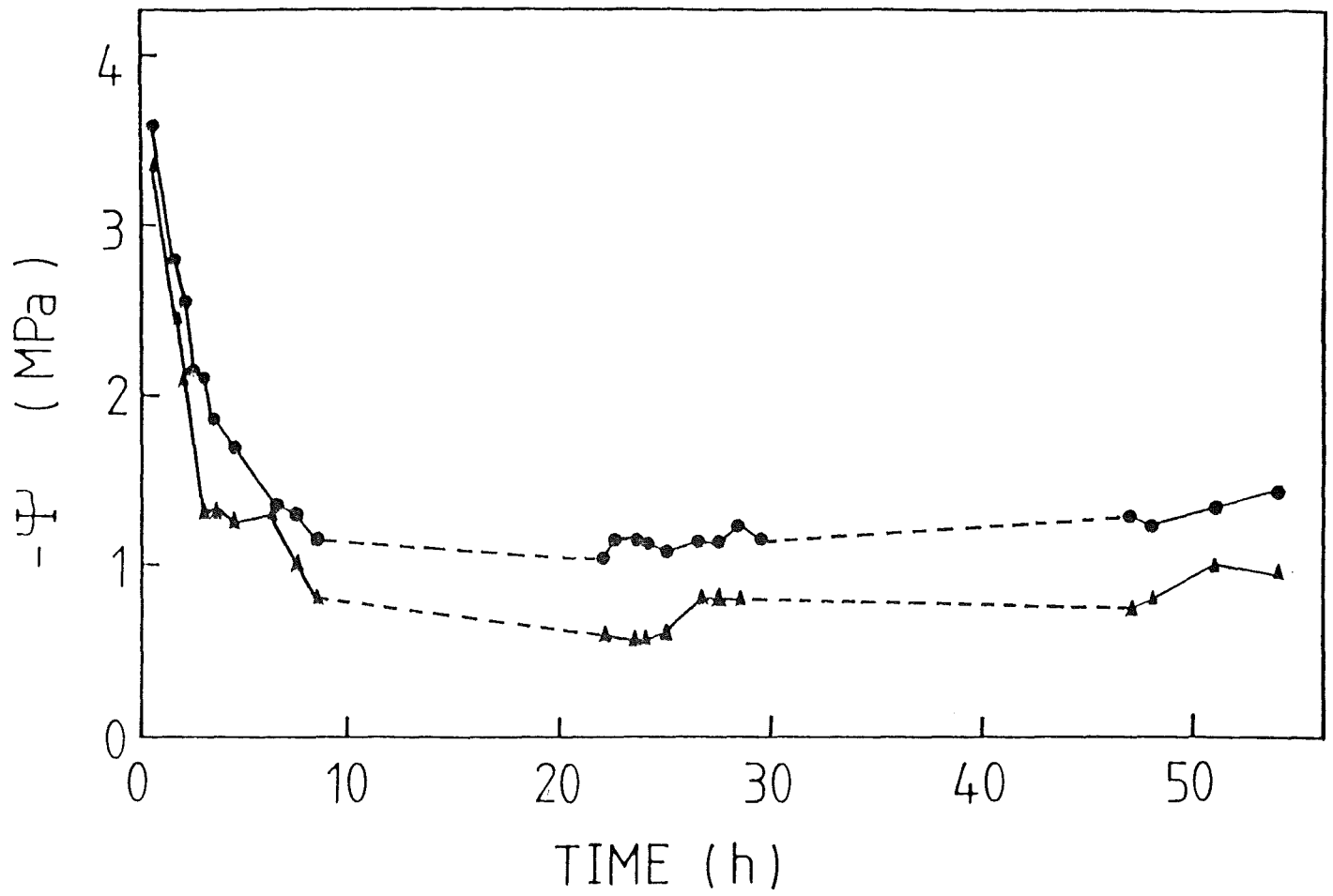


Figure 2. Equilibration of Rhododendron leaf discs in the new psychrometer chambers. Both leaf discs were cut from a single leaf of balance pressure 0.84 MPa.

sodium chloride solutions of known osmolality (Weast, 1981).

The refractive index of expressed sap samples was measured using an Abbe Refractometer (Otago Optical Works, Tokyo, Japan) and the refractive index expressed relative to that of water.

2.3.3. The pressure chamber

Two Scholander-type pressure chambers (Scholander et al., 1964 ,1965) of internal dimensions 55 x 120 mm and 30 x 300 mm were used. Two Bourdon pressure gauges with ranges 0-6 MPa and 0 4.14 MPa were used interchangeably with the two pressure chambers. The gauges were calibrated at the beginning of the project and checked against each other at intervals during it.

The pressure chambers were initially pressurised with air from diving cylinders. A change to oxygen-free nitrogen was made later to minimise adverse effects of high oxygen partial pressures on cells (Tyree et al., 1973; Jones Pers. comm.). However, Tyree et al. (1973) also warn of the effects on leaves of long periods of exposure to nitrogen at high pressure. Experiments testing for adverse effects of using nitrogen or air in the pressure chamber when making prolonged or repeated measurements of balance pressures are described in section 3.3.

Water loss from samples in the shorter pressure chamber was minimised by lining the internal walls of the chamber with damp paper towel. The small internal diameter of the taller chamber required that chamber humidity be maintained by admitting gas through a pad of wet paper towel over the gas port as paper towel on the chamber walls interfered with the mounting of samples in this chamber.

When deriving the relation of balance pressure to relative water content (section 2.4.1) leaves were not enclosed in plastic bags when inside the pressure chamber. This omission speeded work but, as leaf weights were measured upon removal from the chamber soon after measuring balance pressure,

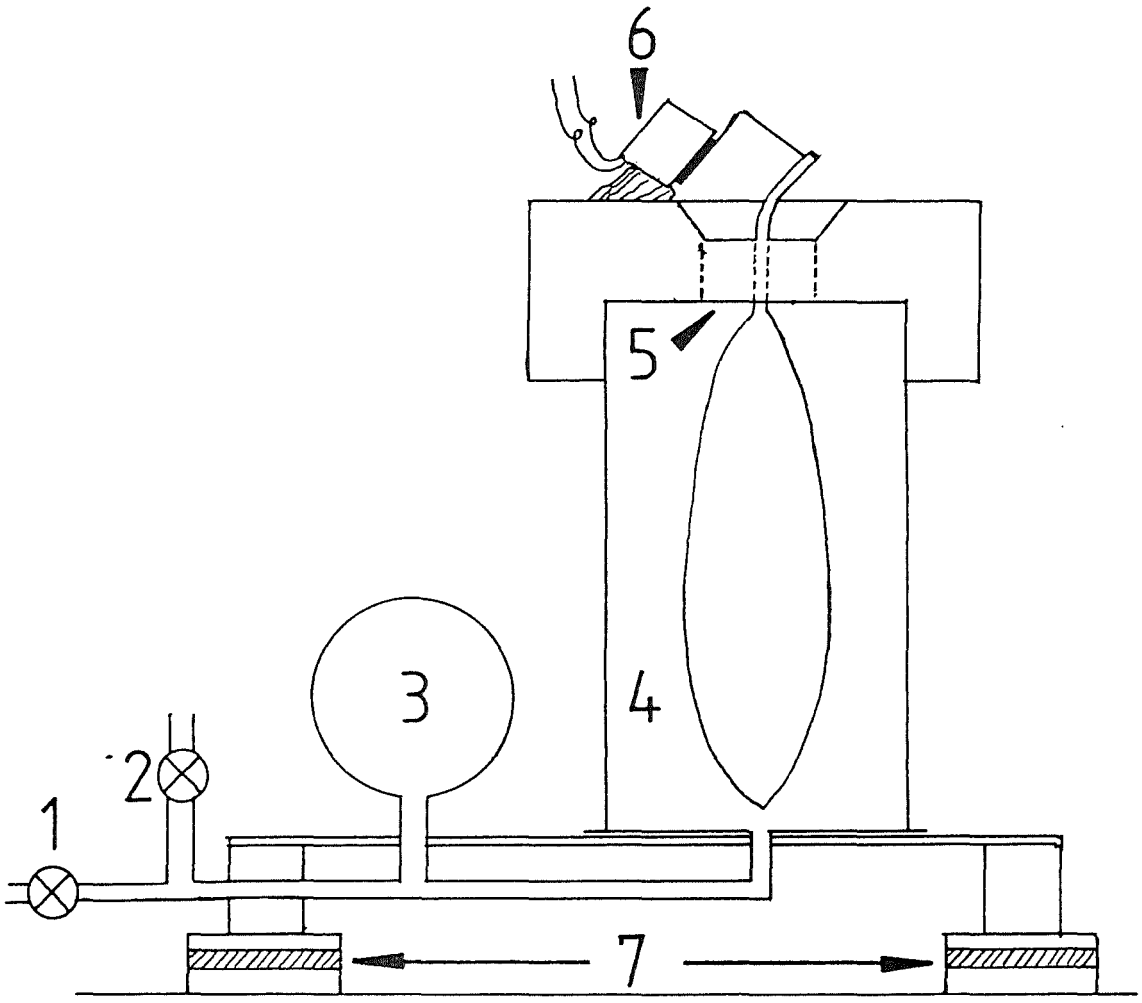


Figure 3. The use of the pressure chamber to control sap tensions in a leaf during acoustic experiments. The leaf was positioned in the pressure chamber (4) and the acoustic detector (6) inserted into the petiole either above or below the rubber bung (5) sealing the leaf into the pressure chamber. Damping materials were placed under the pressure chamber (7) and the acoustic detector to reduce noise. Sap tensions in the leaf could be altered by increasing or decreasing chamber pressure by known amounts by use of inlet and exhaust valves (1,2) and the pressure gauge (3).

without allowing large weight changes due to evaporation.

When measuring balance pressure for purposes other than that mentioned above, leaves were sealed into small plastic bags, prehumidified by breathing into them, before mounting in the pressure chamber to prevent changes in water potential between sampling and obtaining the balance pressure (Jones and Higgs, 1979).

The length of stem or petiole protruding from the pressure chamber was kept to between 5 and 10 mm to minimise errors caused by non-pressurisation of part of the sample (Slavik, 1974). Samples were sealed into the pressure chamber by inserting the petiole or stem through a slit cut into the rubber bung sealing the sample port in the lid of the pressure chamber. Gas leaks around the sample where it passed through the seal were prevented by caulking the space around the petiole with a commercial sealing compound. Blu-tak (Bostik Ltd., Leicester, UK) and Terostat (obtained from the Grace Container Equipment Centre, Elveden Rd., London, UK) were used for this.

In view of the low water potentials involved in this study, equilibration errors were thought likely if balance pressures were raised rapidly. Pressure was therefore raised at about .005 MPa s^{-1}, a rate which minimized inequilibria arising due to differences with which parts of a sample may exchange water with the xylem (Tyree et al., 1978). After the first expression of sap the pressure was released by 0.1 - 0.2 MPa and slowly increased again. When the pressure at which sap reappeared at the cut surface was the same on successive measurements the balance pressure was noted. Balance pressures were measured to within ± .01 MPa although end points were less definite in some species, particularly the herbs.

2.4. Relative Water Content (RWC)

Relative water content (RWC) was defined in the same way as the relative turgidity of Weatherly (1950)

$$\text{i.e.} \qquad RWC = \frac{LW - DW}{TW - DW} \times 100\%$$

where LW = leaf weight measured during an experiment, TW = turgid weight is leaf weight at full turgor (achieved by overnight hydration) and DW = dry weight (after drying for two days at 363K).

2.5. Sap flow in the xylem

2.5.1. Sap flow in stem segments

A quantitative study of the effect of cavitation on the ability of xylem to conduct sap was made using stem segments of Rhododendron and Ricinus.

The relative conductivity (K) of the stem segment was defined as follows (Heine, 1971) and has the units (m^2)

Eqn. 5. $$K = \frac{Q.l.\eta}{\Delta P.A}$$

in which

A = total cross-sectional area of xylem (m^2)

l = length of liquid flow path (m)

η = liquid viscosity (Pa s)

ΔP = pressure difference causing flow (Pa)

Q = liquid flux entering or leaving the stem segment ($m^3 s^{-1}$).

2.5.1.a. Calculating K

A. Photographs of the apical end of the stem segments were taken and xylem area measured by weighing cut-out traces of the xylem in the cross section. It was thought that, as the limiting resistance to flow was most likely to be in the part of the segment with the least xylem, measuring the xylem area at the narrower end of the segment would give the best measure of the effect of xylem area on flow.

l. Liquid flow was assumed axial, making l equal to the length of the stem segment, which was measured to ± 0.2 mm.

η. As water was used as the permeating fluid viscosity could be taken from standard tables, assuming that eluates from the stem and matric forces in the smaller pores through which the water flowed had negligible effect on viscosity. As the viscosity of water is temperature dependent, experiments

were conducted in a growth room in which temperature was controlled to 25 ± 1C At this temperature the viscosity of pure water is 0.8904×10^{-3} Pa s^{-1} (Handbook of Chemistry and Physics, Weast (1981).

ΔP. A simple apparatus was constructed by which a constant head of water could be supplied to the stem segment whose permeability was being measured. The apparatus included an inlet potometer for measurement of Q (see below). The apparatus is illustrated in figure 4.

A constant head of water was maintained by a float tank receiving water from a header tank at a higher level. Hydrostatic pressures were measured by two water manometers connected near the inlet and outlet ends of the stem segment. The manometers were of 2mm internal diameter glass tubing held against a metre rule. The position of the meniscus in each manometer could be read to ± 0.5mm against the wooden ruler to which the manometers were secured. ΔP was calculated from the difference in height between the menisci. Hydrostatic heads were typically 0.75 to 0.77 m.

Q. Flow of water through the stem segment was measured on the inlet side by a bubble potometer taped to a metre rule. The position of the bubble could be read to ± 0.5mm if flow was slow and with somewhat less precision if flow was rapid. Bubble position was adjustable by two water-filled syringes mounted in T-pieces at either end of the potometer. The potometer was calibrated as recording a volume passage of 7.59×10^{-9} m^3 of water per metre of bubble movement. A similar potometer was originally installed to measure flow from the stem segment. However, it was found that eluates from the stem quickly contaminated the narrow potometer causing the bubble to stick, resulting in intermittent rather than constant flow. Consequently efflux was measured by collecting water in a phial on the weighing pan of a milligram balance. A layer of light oil on the surface of the water in the phial prevented evaporation.

Connections between tanks, potometers and the collection phials were of 5mm Tygon tubing. Joints were sealed by stretching latex tubing over them. Stem segments under test were also connected into the apparatus by latex tubing stretched over the ends of the segment and the corresponding influx or efflux ends of the Tygon tubing.

Glassware was cleaned at intervals by washing with chromic acid and the Tygon tubing washed with 70% ethanol.

The apparatus was thoroughly flushed with distilled and filtered water after cleaning and reassembly.

The output end of the stem segment could, if required, be connected to a vacuum source rather than to the collection phial. In this way water flow rates at pressure differentials greater than those attainable by hydrostatic head alone could be measured. Vacuum was measured by a mercury manometer to ± 0.5 mm and the total pressure differential causing flow calculated as the sum of hydrostatic head at the inlet side of the segment and the vacuum on the outlet.

2.5.1.b. Purification and degassing of water. Booker (1977) has listed the steps that must be taken in the preparation of water to be used for permeability experiments if reliable results are to be obtained.

Water was therefore prepared by the method of Edwards (1980) which satisfies the requirements laid down by Booker (1977).

Distilled water under a 2m head was passed through two inline filters. The first (Whatman Gamma 12-03) prevented passage of particles larger than 0.3 μm and the second, a nitrocellulose millipore membrane (Sartorius Type SM11309) supported on a closely drilled perspex plate, removed particles down to 0.1 μm. The filtered water was stored in a glass vessel until used.

Immediately before use the water was degassed by sonication under vacuum. The water, in a 5 litre Buchner flask, was held under vacuum by an Edwards high vacuum pump (Edwards High Vacuum Ltd., Crawley, England) with the tip of

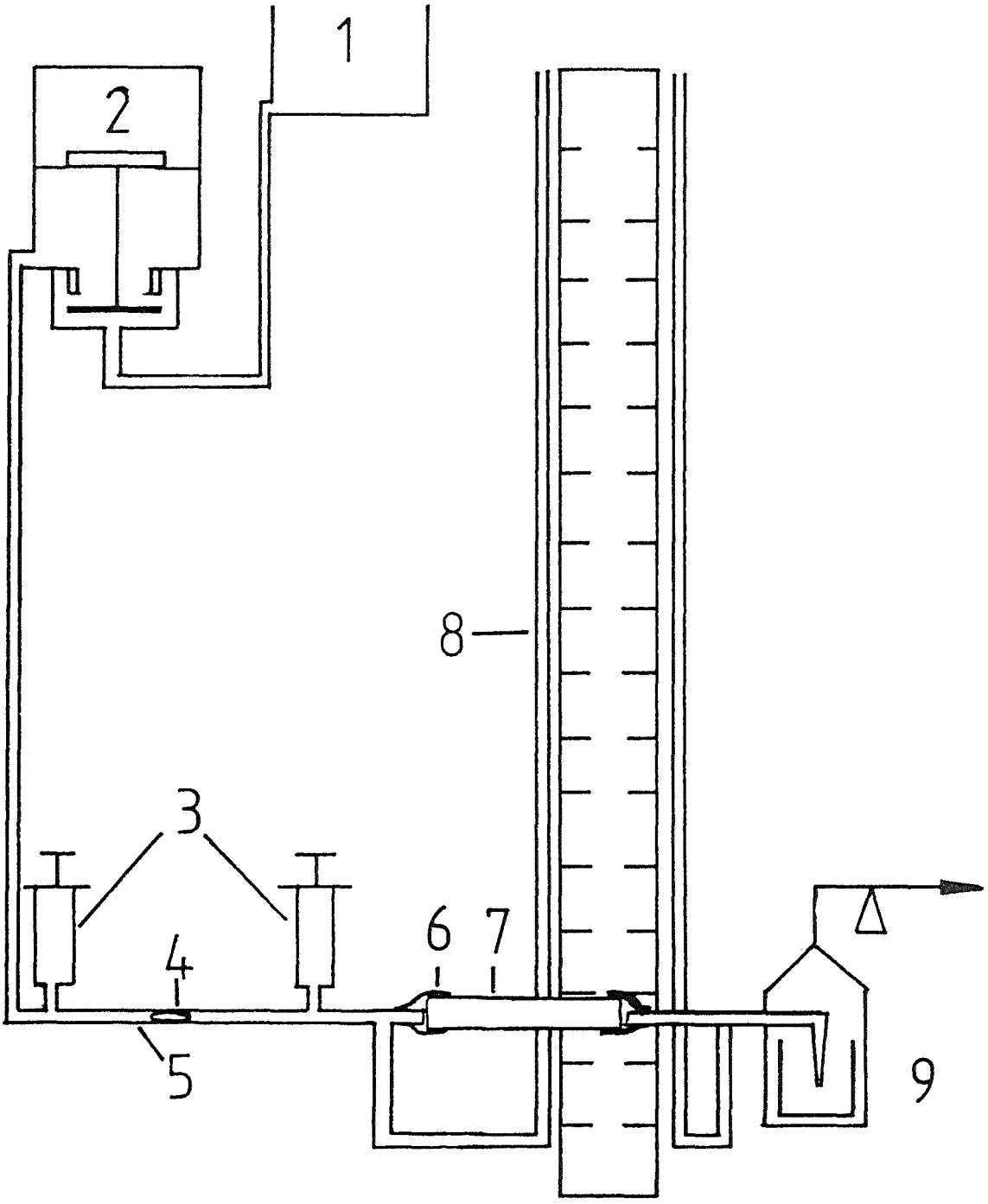


Figure 4. Constant head apparatus for measuring the relative conductivity of stem segments. A reservoir (1) supplies water to a flat tank (2) which maintains a constant hydrostatic head of water. The flow of water into a stem segment (7) mounted in the apparatus by latex rubber sleeves (6) is measured by observing the movement of a bubble (4) in a potometer (5). The position of the bubble is adjusted by water filled syringes (3). The pressure drop across the specimen is measured by inlet and outlet manometers (8) and the flow from the sample is measured gravimetrically (9).

the ultrasonic generator probe (MSE model N114 ultrasonic generator) passed through the rubber bung sealing the flask and in contact with the water. Sonication at 25 kH2 caused immediate considerable frothing which declined after a few minutes. The water was considered degassed when only occasional bubbles formed in the water.

Because of the large surface area of water exposed to air in the float tank air quickly redissolved into the degassed water. However, so little water was actually used in each experiment that the 20-25 x 10^{-6} m^3 of water held separate from the air in the Tygon tubing (and therefore not absorbing gases) between the float tank and stem segment was sufficient to last for several hours. The apparatus was refilled with degassed water after at most three hours of use.

2.5.1.c. Preparation of stem segments for measurement of K. Segments were cut underwater from shoots and 40mm trimmed to remove emboli from ends which had been exposed to air. The bark was stripped from the segment, the ends pared smooth with a razor blade and the segment connected into the permeability apparatus. The connections were checked for leaks or contained air and the exposed parts of the stem heavily smeared with lanoline after the segment had been lifted from the water.

2.5.1.d. Determination of water flow paths. After measurement of Q, the path of water movement in the stem was stained by injecting decolorised basic fuchsin (section 2.8) into the permeating water. The dye was allowed to flow through the stem for 10 minutes before the segment was removed from the apparatus.

After photographing the apical end of the segment for determination of xylem area the segment was sectioned to determine the pattern of dye staining in the xylem.

2.5.1.e. The flow of water through stems at increasing pressure differentials. By use of increasing vacuum the flow of water into stem segments at pressure differentials between 0.007 and 0.05 MPa could be measured.

Flow at higher pressure differentials was measured using the pressure chamber.

A stem segment was mounted in the pressure chamber such that 80-100 cm of its length was inside the chamber and dipped into a phial of distilled and filtered water. Collection of water from the end outside the chamber was facilitated by a length of latex tubing fixed over the end and emptying into a small phial. Water was collected over one or two minute intervals as the chamber pressure was raised in increments from 0 to 4.14 MPa. Exudation volumes were determined by weighing the collection phials before and after a collection.

2.5.2. Potometric measurement of the effect of cavitation on sap transport

Water uptake into shoots was modelled on the van den Honert electrical analogy (1948) as was water flow through stem segments in section 2.5.1. The analogy applied was that of a capacitor charging through a resistance and not simply the flow of current through a resistance as in 2.5.1.

The major difference in calculation of K for the xylem of shoots compared to that of stem segments concerned the measurement of the pressure difference (ΔP) causing flow. Other differences concerned the length and xylem cross-sectional area through which flow was occurring.

Because the stems of Rhododendron shoots taper from base to apex and as the leaves (postulated as the sinks to which water moved, see below) were distributed along this tapering stem, a meaningful value for the area of the xylem cross-section was thought to be unobtainable.

A modified form of the equation for calculation of relative conductivity which omitted the area factor was proposed to measure the ability of shoot stems to conduct water. A new term, K^1, was defined as

Eqn. 6. $K^1 = \frac{Q.l.\eta}{\Delta P}$

K^1 is therefore in units of m^4 (i.e. $m^2 \times \frac{l}{l_{m^2}}$).

The symbols have the same meaning as for the calculation of K in section 2.5.1. although, because shoots rather than stem segments were used, their measurement was slightly different.

2.5.2.a. Calculation of K^1.

l. The distance l could not be measured as the length of the stem as the major sinks for water taken up, the leaves, were distributed over a considerable distance along it. What was thought to be a reasonable nominal value for l was obtained by measuring the distance between the cut end of the stem and the middle of the zone of petiolar insertions.

η. These experiments were conducted in a laboratory lacking temperature control. Temperatures varied between 293 and 298K during experiments. Consequently, an approximate value of 10^{-3} Pa s was used in calculations of K^1.

ΔP. The electrical analogy proposed to describe water uptake by a shoot was that of a capacitor charging through a resistance. In such a system the voltage difference between voltage source and capacitor decreases according to an exponential decay of the form

Eqn. 7. $E = a * e^{-b_1 t}$

The pressure differential between shoot protoplast and the free water supplied to the shoot in potometric experiments was similarly described. However, because of the complexity of the structure of the shoot two different capacitances charging through different resistances were postulated (section 4.3).

Q. The long arm of the potometer was held against graph paper ruled at millimetre intervals. The rate of water uptake by the stem was then found by using a stop-watch to time the movement of the meniscus in the potometer over the graph paper. The position of the meniscus could be determined to ± 0.5 mm at normal flow rates and somewhat less certainly at very high fluxes. Potometer calibration was 3.8×10^{-9} m^3 for each metre of meniscus movement.

2.5.2.b. Measuring water uptake by shoots and leaves. Shoots were prepared for mounting on the potometers by cutting 40mm underwater from the end of the stem to remove emboli and the bark cut back for 20mm more to leave a smooth xylem surface against which a good seal could be made. The cut surface was pared smooth with a razor blade. The exposed xylem was connected to the potometer by slipping a short length of latex tubing over both the short arm of the potometer and the exposed end of the xylem. All operations were carried out under filtered water (section 2.5.1.b) to prevent air entry to the xylem. The shoot had previously been sealed into a plastic bag to prevent transpiration. Only the end of the stem protruded from the bag. The shoot was now taped to a flat support to prevent movement. If desired, the support could be moved vertically so that the shoot could be dipped into a waterbath.

Experiments were timed from the first underwater cut.

Leaves were mounted similarly after cutting from the stem underwater. The arrangement is shown in figure 5.

2.5.3. The effect of cavitation on sap transport in intact plants

Potted Rhododendron plants in a glasshouse were used for this study.

The plants were subjected to slowly developing sap tension by withholding water over a period of 2-4 weeks. Plants were divided into three classes, non-cavitated, cavitated, and cavitated and recovered (by restoring water at field capacity by twice daily watering for three or five days).

Relative conductivity of the xylem was measured on excised stem sections

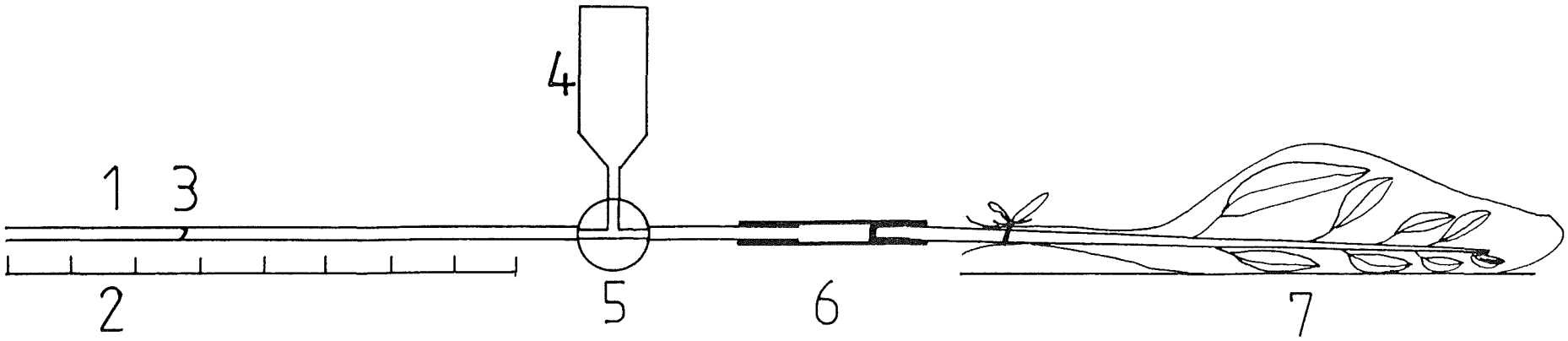


Figure 5. Potometer measurement of the rate of water uptake by shoots. Movement of the miniscus (3) in the long arm of the potometer (1) over graph paper (2) was used to measure the rate of water uptake by a shoot connected to the potometer by a rubber sleeve (6) and enclosed in a plastic bag (7) to prevent transpiration. The long arm of the potometer can be refilled from the reservoir (4) without admitting air to the xylem by using the three-way tap (5).

by the method of section 2.5.1.

The effect of cavitation on the ability of the plant to maintain its water status was investigated by comparisons of the leaf water potentials and stomatal conductance of transpiring potted plants in each of the three classes above. These indicators of plant water status were measured at two hourly intervals over a sunny day in July 1982.

Stomatal conductance was measured using a Li-Cor Diffusive Resistance Meter (Lambda Instruments Corp., Lincoln, Nebraska) calibrated in the laboratory using a water bath as set out in the instrument manual.

Water potential was measured by the pressure chamber (section 2.3.3). Sap tension was measured by taking the balance pressure of leaves which had been enclosed in small plastic bags wrapped in aluminium foil (*Turner, 1981) for at least twenty minutes before sampling.

Relative humidity and air temperature were measured at intervals by measurement of wet and dry bulb temperatures. Continuous records of relative humidity and air temperature were made by a thermohygrograph (Casella, London) on the glasshouse bench.

Radiant energy flux was measured by a Kipp and Zonen integrating solarimeter (Kipp and Zonen Ltd., Delft, Holland) situated on the roof of the laboratory some 50 metres from the glasshouse.

2.6. Gas penetration of pit membranes

Sap tension will be rapidly released when a gas phase appears in the conduit. The rapid release of sap tension will occur irrespective of whether the gas phase arises within the conduit or enters from outside through pit membranes. Clicks detected by the acoustic technique will occur in either case as they are (supposedly) caused by elastic recoil of the cell walls.

Experiments were therefore conducted to investigate the penetration of pit membranes by gas under pressure with the aim of determining whether clicks occurred as the result of cavitation of the xylem sap in a conduit or by gas entry through the pit membranes.

Gymnosperm wood is unsuitable for such studies as aspiration of the tori of the bordered pits will close the pit aperture with a non-porous plug and so prevent gas entry (Dixon, 1914; Bailey, 1916; Petty, 1970).

Angiosperm wood lacks the complex bordered pits of the gymnosperms. Gas entry into conduits is prevented by retention of sap in the very small pores of the pit membranes (Dixon, 1914).

Rhododendron stems were used for these experiments as

i) the sap tension at which clicks occur was known;

ii) the vascular pits are of the angiosperm type and lack tori;

iii) xylem vessel lengths were sufficiently short (section 3) that stem segments with only a very few conduits lacking at least one pit membrane were of convenient length for use in the pressure chamber;

iv) the stems were strong enough to resist crushing in the pressure chamber seals and did not include non-xylary spaces which might pass gas.

2.6.1. Gas permeability of stems. The retention of sap in the pit membranes against high gas-sap pressure differentials can be tested for by applying gas under increasing pressure to one side of a wet pit membrane and noting the rate of gas flow through the membrane. The forcing of sap from the membrane results in pores being opened to the passage of gas with the consequent increase in the measured rate of gas flow.

Unbranched shoots of up to three years growth were collected and hydrated as described previously (section 2.1.1.). After pretreatments (see below) the stems were prepared for measurement as follows. The terminal leafy portion of the shoot was removed and the bark and leaves of previous seasons stripped from the stem. The ends of the stem section were planed smooth using a razor blade.

The stem was weighed and then wrapped in Sellotape before being overlaid by wet paper towel secured by more tape to restrict evaporation. The stem was mounted in the pressure chamber with 40-50 mm of stem inside the chamber. Restricting the amount of stem inside the chamber was necessary to minimise Hammel-type sap expression from living tissues subjected to pressure inside the chamber.

Nitrogen gas entering the chamber was humidified by passage through a pad of wet paper towel over the gas port.

The resulting gas flow through the stem was measured as shown in figure 6. Gas emerging from the stem was collected into a flexible tube connected to the end of the stem by a length of latex tubing.

Gas flux rate was determined by noting the time taken for the collected gas to displace a set volume (5×10^{-4} m^3) of saturated brine solution from an inverted measuring cylinder. Gas was channelled to the top of the cylinder through a glass tube to prevent bubbling which made observation of levels in the cylinder difficult. A Mittyvac (Neward Enterprises, Cucamonga, California) hand operated vacuum pump was used to restore the brine to the top mark between experiments. A three-way tap and independently operating vents enabled brine levels to be adjusted without disturbing the connections to the stem and rapid switching of gas flow from vent to the measuring cylinder.

The resistance of the system to gas flow was very low by comparison with even the lowest of the stem resistances and could not be measured under even the smallest pressures used in experiments.

The rate of gas flow through the stem was expressed as units of volume of brine displaced per second (m^3 s^{-1}) by measuring the'transit time' taken for the brine levels to fall between marks on the measuring cylinder being used in this calculation.

Gas flow through the stem was measured at increments of increasing and decreasing pressure over one or more cycles for each stem.

2.6.2. Expression of sap from xylem conduits

Gas penetration of pit membranes not only increases the gas permeability of the stem; entry of gas into a conduit will displace the water already in that conduit. If the living tissues of that stem do not have large water deficits, or are separated from the sap in the xylem conduits by high resistances to lateral flow, most of this displaced water will be expelled from the low pressure end of the stem. Measurement of the volume of water expressed for each increment of pressure will reveal at what pressure gas enters the xylem conduits.

Stems were mounted in the pressure chamber as described, and chamber pressure increased in 0.18 or 0.35 MPa increments.

Immediately that pressure was raised, a plastic phial containing paper tissue was placed on the exposed end of the stem to absorb the expelled water. The tubes were changed at two-minute intervals and immediately capped by clipping a larger, snug-fitting phial over the open end to prevent evaporation. The capped phials were then weighed and the amount of absorbed water found by subtraction of the tare weight of the phial and its cap.

Sap collection was continued until the collection over two minutes was less than 5% of the running total for that pressure increment. Evaporation of collected water from the open phials and from the stem (section 3.8.3), particularly at high pressures, made continuation of collections past this time counter-productive.

The amount of sap collected was summed for each increment and expressed as a percentage of the total collection volume for that stem.

The effect of changing surface tension on gas penetration of pit membranes

Surface tension in aqueous solutions is lowered by the addition of surfactants, such as detergents and soaps, or organic solvents, such as alcohols. It was thought that adding strong detergents to the transpiration stream might have serious deleterious effects on the membranes of living cells in the stem. Cell debris and contents which might accumulate on pit membranes

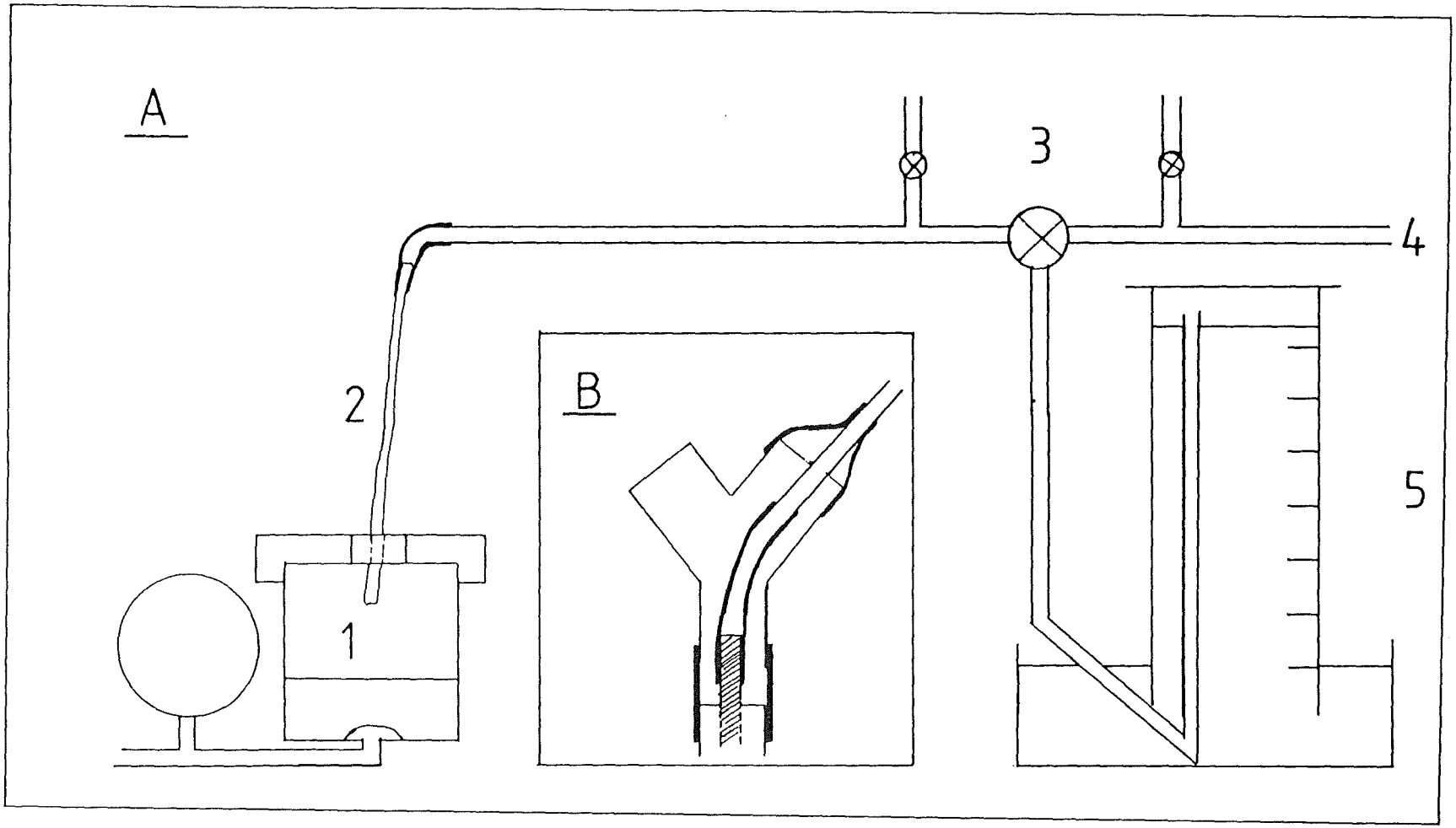


Figure 6. A Measuring the flow of gas through stems. Gas passing through a stem in the pressure chamber (2) was collected over brine in an inverted measuring cylinder (5). By use of two and three-way taps (3) and a vacuum line (4) the measuring cylinder could be quickly refilled with brine.

B (Inset). Separation of gas flowing from the pith (hatched area) and xylem (plain area) of Rhododendron stems.

could be released. Alcohols, especially the higher alcohols, were considered probably to be less toxic in their effects on plant tissues. A range of surface tensions could also be readily obtained by adjusting the concentration of alcohol in the solutions.

Ethanol has been shown to have limited toxic effects on the xylem structure, at least in the short term, by Mishagi et al. (1978). However, its volatility may lead to rapid changes in concentration when large volumes of gas are passing in nearby conduits in stems. N-butanol was therefore selected to reduce sap surface tension. The surface tensions of the solutions used are given in table 1.

Table 1. Surface tensions and approximate water potentials of permeating solutions transpired by shoots. Values of surface tension are from the International Critical Tables, Washburn (1926) for a temperature of 25°C.

	Solution			
	Water	5.4% NaCl	10% N-butanol	4% N-butanol
Surface Tension (Pa m x 10^{-3})	71.2	74.4	27.0	48
Water Potential (MPa)	0	-4.37	-	-

These liquids were introduced into the xylem by substituting them for water in the transpiration stream of shoots transpiring on the laboratory bench. A three-hour transpiration period was settled on as being sufficient for perfusion of the solution without being so long that possible toxic effects of the solutions would be severe.

2.7. Determination of Conduit length

A knowledge of the length of xylem conduits in the stems and leaves of plant material used to establish the effect of cavitation on sap transport was necessary, for instance in the selection of the lengths to be trimmed from

bench dried shoots to remove air embolised conduits. The knowledge is also useful in interpreting the results of these and the acoustic experiments.

Conduit length was determined by injection of Indian ink into xylem conduits.

Indian ink was filtered three times through Whatman number one qualitative filter paper and diluted to 1/40 of its original concentration. This dilution gave a suitable volume of filtrate with which to conduct experiments, reduced the probability of clogging of conduits as a result of water loss through walls rather than the ends of the conduits (Handley, 1936), and reduced the likelihood of cell damage by preservatives in the undiluted ink.

The size of particles in the ink was measured from photomicrographs taken using a transmission electron microscope (plate 1). Maximum and minimum particle dimensions measured from these photographs were 46 μm and 17 μm respectively.

Shoots or leaves were hydrated (section 2.1) and the dilute ink suspension supplied to the cut petiole or stem to ascend with the transpiration stream. After injection material was hand-sectioned at intervals and the number of ink-containing conduits counted using a microscope.

2.8. Determination of Sap Flow Paths

The conduits conducting sap were determined by injection of decolorised basic fuchsin.

Basic fuchsin was prepared by addition of 10 ml of 10% sodium meta-bisulphite to 100 ml of 0.1% aqueous basic fuchsin (Talboys, 1955). The solution lost its red colour over several hours but a light yellow colour (due to impurities in the dye - Coleman, 1938) remained.

The solution was stored in an airtight jar. A light precipitate formed with time. Care was taken not to disturb this precipitate when taking dye for infusion into stems.

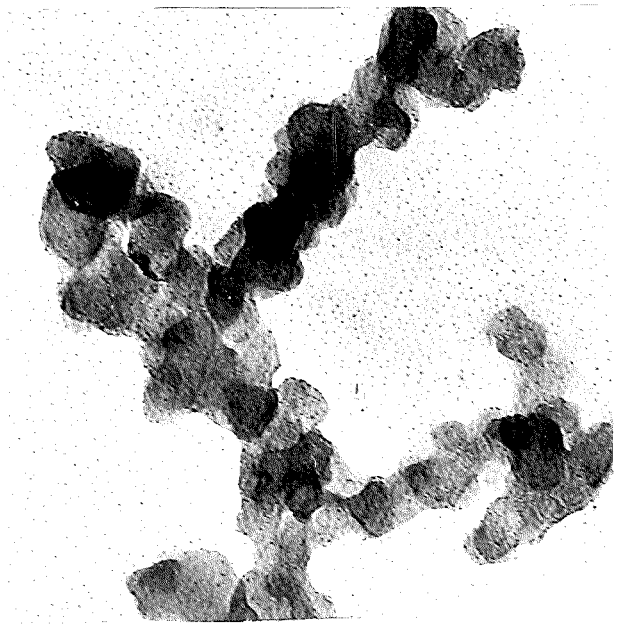


Plate 1. Particles of Indian ink from the filtered and diluted suspension used to measure length of xylem conduits ($x10^5$).

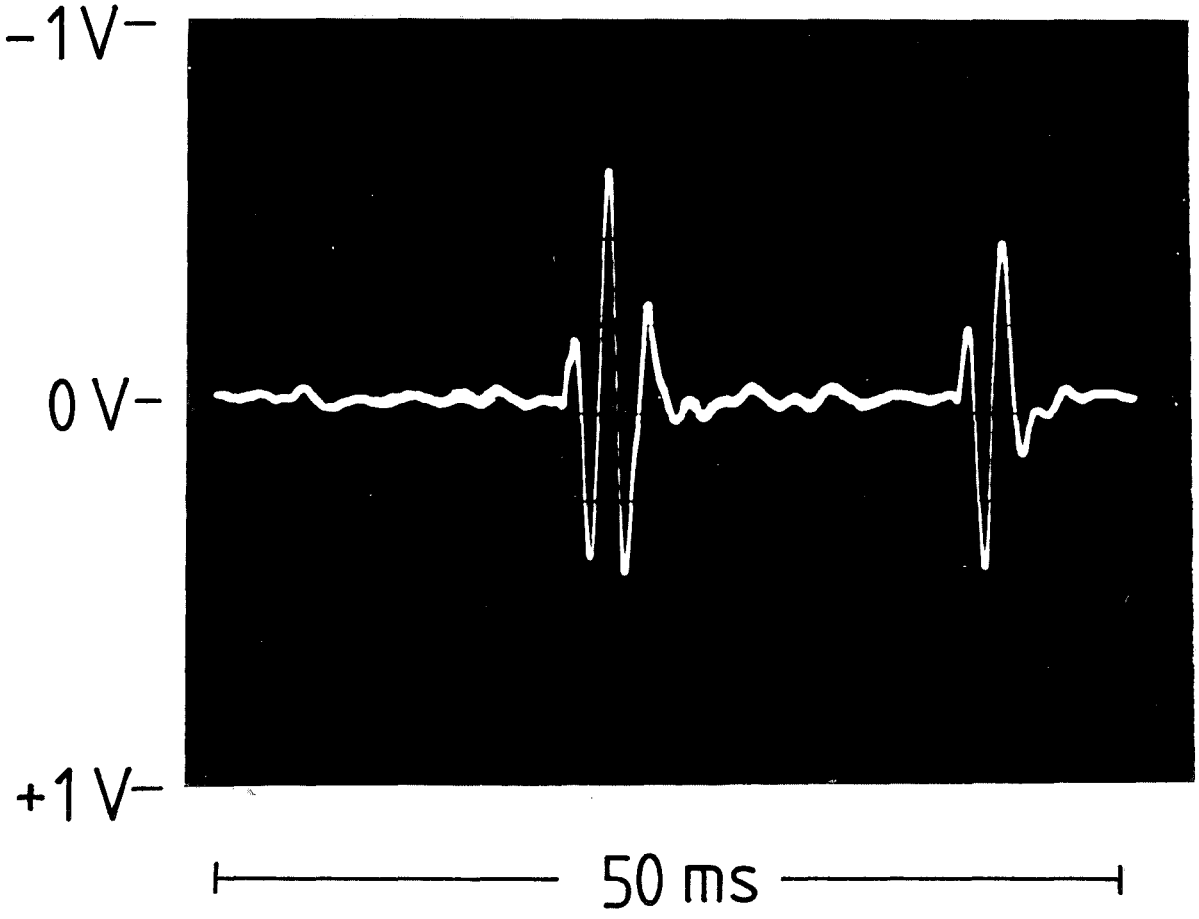


Plate 2. Oscilloscope traces of clicks detected in Rhododendron leaves by the acoustic detector fitted with ceramic transducers.

2.9. Xylem anatomy

Petioles from leaves similar to those used in acoustical experiments were stored in 2.5% formalin (Dimond, 1966) and embedded in paraffin wax after dehydration through an alcohol series terminating in N-butanol (Johansen, 1940).

25 μm thick transverse sections of these petioles were cut by rotary microtome, the wax removed with xylol and the sections stained with toluidine blue. Photomicrographs were taken using a Zeiss Photomicro-system 2 (Carl Zeiss, Oberkochen, West Germany) camera microscope.

2.10. Computation of results

Experimental data were entered onto files held on the University of Edinburgh Regional Computing Centre ICL2900 computer at the Kings Buildings, Edinburgh.

Statistical calculations were made by calls to the MINITAB package (Minitab Project, Pennsylvania State Univ., Pennsylvania). More complex curves were fitted by NAG (Numerical Algorithms Group Ltd., Oxford, UK) routines as in the programme described by Hipkins (1978).

Chapter 3. Cavitation and Sap Tension

3.1. Introduction

The acoustic technique and the pressure chamber were used to investigate the relationship between cavitation and sap tension in detached leaves and shoots of a number of species of herbs, shrubs and trees.

In the past investigations into the occurrence of cavitation of the xylem sap have been based on observations of (a) the presence or absence of gas in xylem conduits or (b) the conduction of dyes in the xylem. These methods were discussed in the introduction (Chapter 1).

Two techniques are now available which, by their combination, allow the occurrence of cavitation to be monitored in almost undisturbed xylem, and the sap tension at which cavitation occurs to be found. These are the acoustic technique (Milburn and Johnson, 1966; Milburn 1973a) and the pressure chamber (Scholander et al., 1964,1965) respectively.

The acoustic technique has been used to investigate cavitation of xylem sap in Pyrus (West and Gaff, 1971), Plantago (Milburn and McLaughlin, 1974), Ricinus (Milburn and Johnson, 1966; Milburn 1973a, 1973b) and in Eucalyptus (Crombie, unpublished) and Lycopersicum (Nonhebel, pers. comm.).

The relation of cavitation to sap tension was determined accurately in only two of the above studies. These were for Lycopersicum (Nonhebel, unpub.) and Plantago (Milburn and McLaughlin, 1974).

The work reported in this chapter had two major aims: (a) to accumulate more direct evidence of the link between clicks and cavitation than is already available and (b) to determine the sap tensions at which cavitation occurs in a wide range of species.

The results are presented in five sections, each dealing with a different aspect of the techniques used and the results obtained:-

Section 3.2. The acoustic technique.

Section 3.3. Determination of sap tensions in leaves during acoustic experiments.

Several methods by which the pressure chamber could be used to estimate sap tensions in leaves cavitating on the acoustic detector were evaluated.

Section 3.4. The effect of cavitation on pressure chamber measurements of sap tension and leaf water potential.

Section 3.5. Sap tensions causing cavitation.

The results of acoustic experiments (section 3.2) are combined with those obtained using the pressure chamber (section 3.3) to obtain estimates of the sap tension at which cavitation occurs in the leaves of a number of plants.

Section 3.6. Recovery of leaves, shoots and whole plants from cavitation.

3.2. The acoustic technique

3.2.1. The detection of a 'click' by the acoustic detector

Plate 2 shows oscilloscope traces of clicks detected in a Rhododendron leaf. The traces are typical of many which were viewed in this way and are very similar to those obtained using Ricinus (Milburn and Johnson, 1966).

The waveforms begin abruptly and are irregular in both frequency and duration. Typically a click will consist of one to three high amplitude cycles followed by a rapid decline. The frequency of the 'click' appeared to be governed, at least in part, by the geometry of the detector and was usually lower for detectors fitted with long (20-40 mm) than for those with short (5-10 mm) needles. The frequency of clicks detected in Rhododendron leaves using ceramic transducers was typically 500-600 Hz. This was low by comparison with the 800-1000 Hz for clicks in Ricinus leaves detected using a magnetic detector (Milburn and Johnson, 1966).

Clicks could be detected in samples (leaves or shoots) of all the species (section 2.1) with which experiments were conducted. However, there were considerable differences in the 'loudness' of clicks between species.

Loud clicks were typical of woody species (Acer, Alnus, Eucalyptus,

Fraxinus, Larix and Rhododendron). Softer, but still readily detectable, clicks were found mainly in the soft herbs (Lycopersicum, Phaseolus, Plantago, Ricinus).

Very soft clicks which required special precautions for their detection were found in leaves of Pelargonium and leafy stems of Zebrina. Because of difficulties in detecting clicks in these two species, experiments using them were not continued.

3.2.2. The pattern of click frequency against time

The results of a typical acoustic experiment are shown in figure 7. A Rhododendron leaf was used in the experiment shown. The pattern of results was typical of those found in leaves of all species.

Immediately after mounting, no clicks are detected. After an interval, which varied from only a few minutes to half-an-hour or more depending on the species and experimental conditions, click frequency rose rapidly to a pronounced maximum. A slow decline in frequency then occurred. The time taken until no more clicks were detected took from one to three or more hours, again depending on the species and the experimental conditions. Within this overall pattern of the rise and decline in click frequency, considerable variation in the number of clicks being detected over a short interval could occur.

This general pattern of click frequency with time is similar to that reported for Ricinus (Milburn and Johnson, 1966), Lycopersicum (Nonhebel, unpub.) and Malus (West and Gaff, 1976).

Maximum click frequencies generally varied in accordance with the rate of transpiration by the leaf, being higher if transpiration was rapid than if transpiration was slow.

Leaf weights were measured at intervals during acoustic experiments (section 2) and, by using dry weights obtained later, were then converted to RWC. The decline in RWC of the leaf on the acoustic probe is also shown in figure 7.

From inspection of plots of the type shown in figure 7 it was apparent that

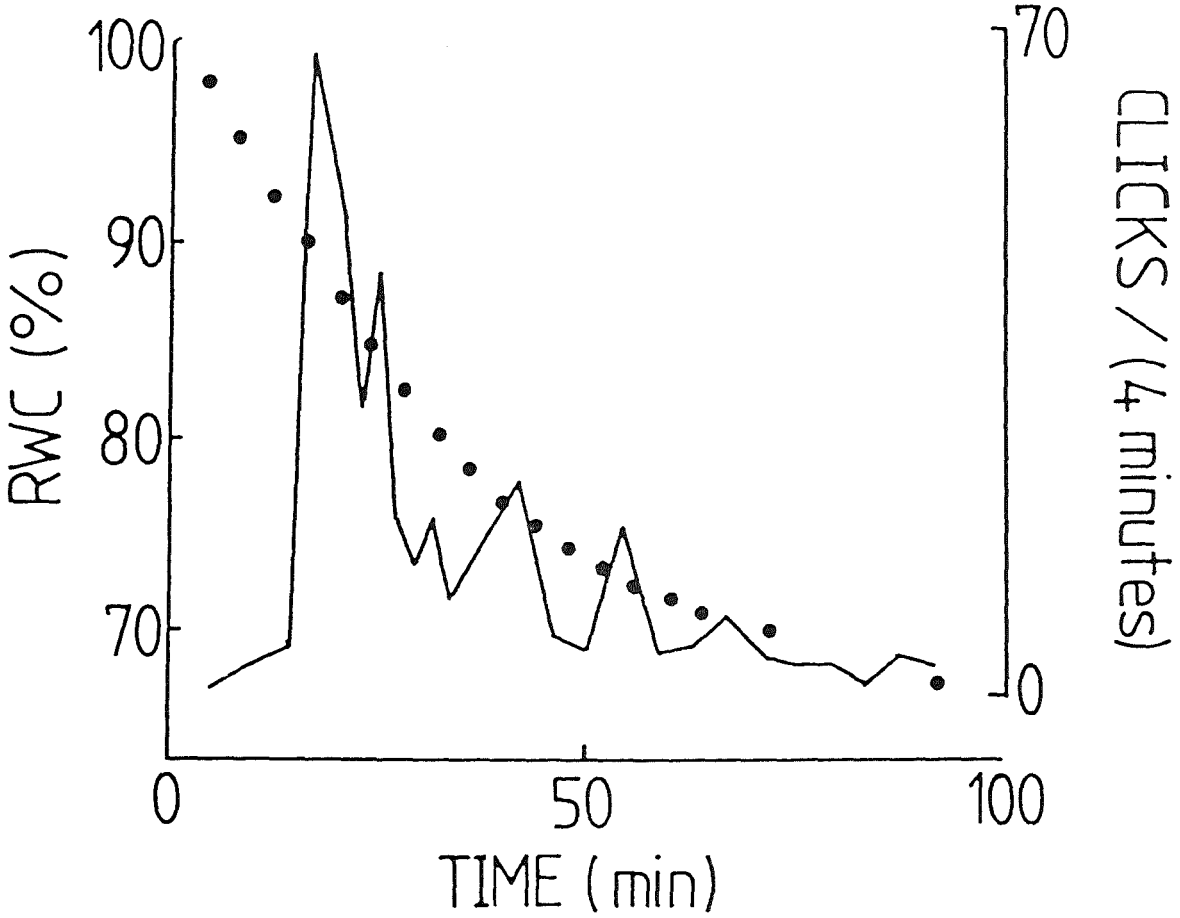


Figure 7. Typical pattern of click frequency (—) and RWC (•) found in acoustic experiments. A Rhododendron leaf was used in the experiment shown.

click frequency was related to the RWC at a particular time as click rate was low until the RWC had fallen by an amount which appeared to be characteristic of each species.

The pattern of click frequency during acoustic experiments was considered to be the only reliable basis for analysis of experimental results. The number of clicks detected was of relatively little importance in analysing experiments because of limitations in the acoustic technique (see below).

3.2.3. Response of click frequency to changes in transpiration rate.

The results of experiments demonstrating the rapid response of click frequency to changes in transpiration rate are shown in figure 8.

Rhododendron leaves and shoots were used in these experiments which were conducted as follows.

a) A Rhododendron leaf was mounted on the acoustic detector and click production monitored as its rate of transpiration was altered by switching off and on again the 60W incandescent lamp used to accelerate leaf water loss.

b) A 200mm long shoot with a cluster of leaves at one end was fitted with an acoustic detector inserted into the xylem of the stem at the other, leafless end. There was approximately 100mm of leafless stem between the acoustic detector and the first leaf of the apical cluster.

Water loss was accelerated by a 60W incandescent bulb. When clicking had become established transpiration was stopped by immersing the shoot in light paraffin oil. Oil was used as the large shoot retained sufficient heat to continue transpiring for a considerable time after the lamp had been switched off (section 3.2.6). Transpiration resumed when the shoot was removed from the oil and oil wiped from the leaves using a tissue.

The appearance of small translucent patches on the leaves indicated that some very limited oil injection of the laminae had occurred.

Click frequency declined rapidly when transpiration by the leaf was slowed

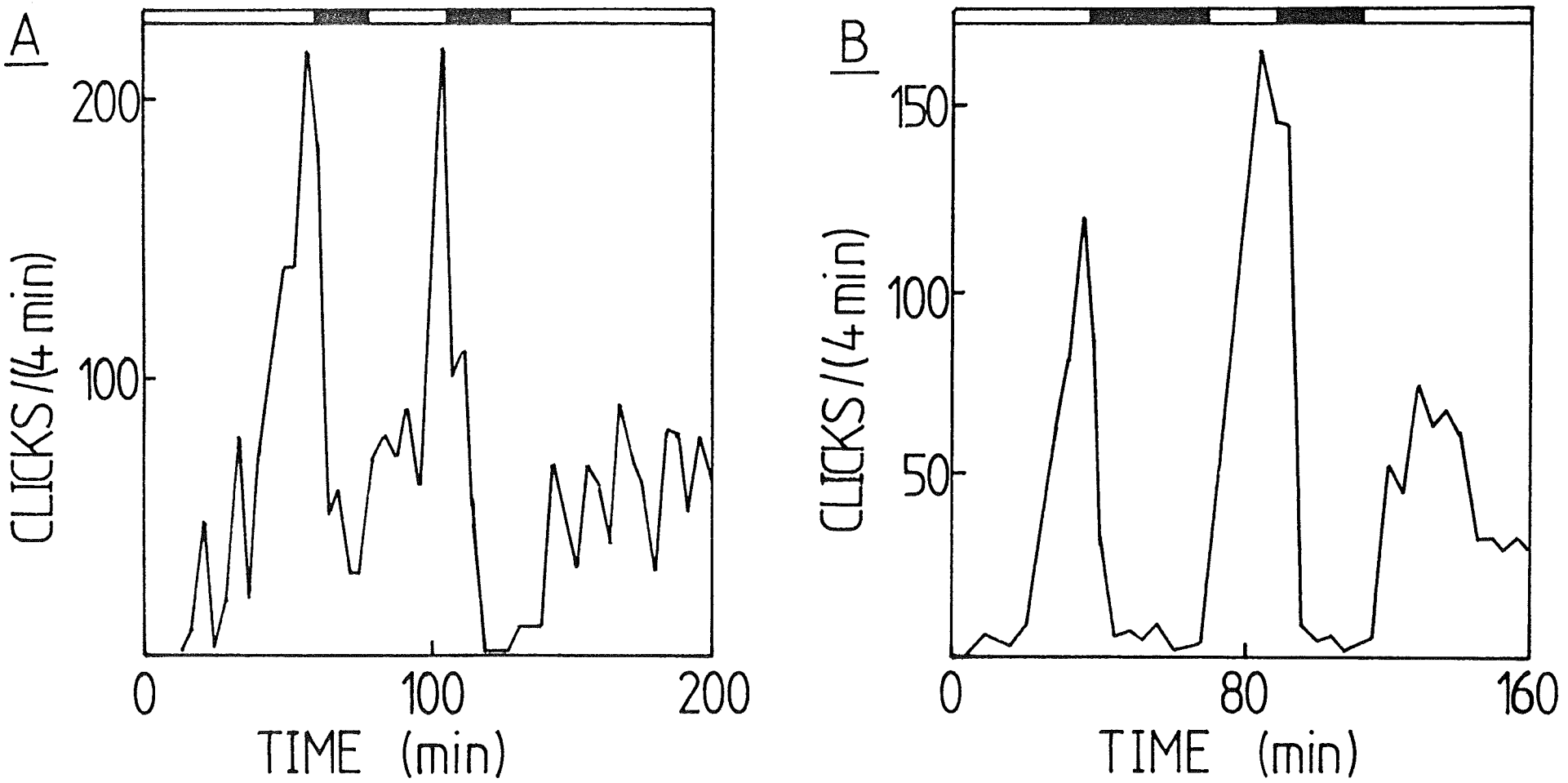


Figure 8. The effect of changes in the rate of transpiration on click frequency in a Rhododendron leaf (A) or shoot (B). Bars at the top of the figures indicate:
White bar - transpiration occurring, being accelerated by incandescent lamp.
Black bar - transpiration held low by switching off lamp (A) or immersing in light paraffin oil (B).

by switching off the lamp (figure 8a) and recovered when the lamp was switched on again. Similar changes were found in the click rate of the shoot when immersed in or removed from the oil (figure 8b).

The rapid response of click frequency to changes in transpiration rate was similar to that observed in Ricinus by Milburn (1973a).

3.2.4. Click pattern and click number

Figure 9 shows the patterns of click frequency against time recorded at different detector sensitivities in an experiment using a single Rhododendron leaf.

It was impossible to use the same detector sensitivities to record clicks in the leaves of all species used in acoustic experiments because of differences in loudness of clicks between species. Moreover, the efficiency of transmission of clicks from the xylem to the needle of the acoustic detector depended on the contact obtained between the needle and the xylem. It was impossible to obtain the same contact when mounting different leaves. Therefore it was necessary to know whether the pattern of clicks detected was altered by changes in detector sensitivity.

To do this, a Rhododendron leaf was mounted on the acoustic probe in the usual manner. The detector output was split and led into both channels of the acoustic detector. The discriminator thresholds of each channel were then set to very different levels, one to record the maximum number of clicks which could be separated on chart records of the experiment, the other to record just sufficient to be able to determine the pattern of click frequency against time. The results of one such experiment are shown in figure 9.

The pattern of clicks detected was very similar for both discriminator thresholds despite very different total click numbers recorded by each during the experiment. This is in accord with the observations reported by Milburn (1973b) and Crombie (unpub.) that in Ricinus and Eucalyptus maculata respectively that number of clicks detected appears to rise exponentially as

discriminator thresholds are reduced. This was attributed to damping of transmission of clicks originating away from the detector mounting site (Milburn, 1973b) and was not thought to reflect cavitation at different specific sap tensions by different conduit populations.

As the pattern of clicks detected during acoustic experiments appeared not to be affected by the setting of discriminator thresholds, these could be set to give a convenient detection frequency for each experiment (section 2.2.3).

Variation in recorded click numbers also occurred as detector output was partly dependent upon the loading of the detector transducer elements. Differences in sample weights and the angles at which the sample hangs from the detector needle will affect the amplitude of signals passed to the discriminator circuits.

In the longer term strains imposed on the transducer elements during mounting and removal of samples, and by overloading during experiments, resulted in a decline in transducer sensitivity. This decline can be retarded by using long needles on the detectors so that samples can be mounted and removed with a minimum of stressing of the transducer elements and by hanging the detector from heavy samples (such as shoots) instead of the sample from the detector so that stress to the transducer elements is minimised.

3.2.5. Clicks detected at different points in a sample

In figure 10 are shown the results of an experiment in which two acoustic detectors were used to record cavitation at different points in a Rhododendron shoot.

As it seemed likely that most of the clicks recorded using a detector inserted into the xylem of a sample (leaf, shoot or whole plant) would have occurred in the immediate vicinity of the detector, these experiments were conducted to ascertain whether the pattern of click frequency against time differed from place to place within the sample.

Rhododendron shoots 150-200 mm long were fitted with two acoustic detectors;

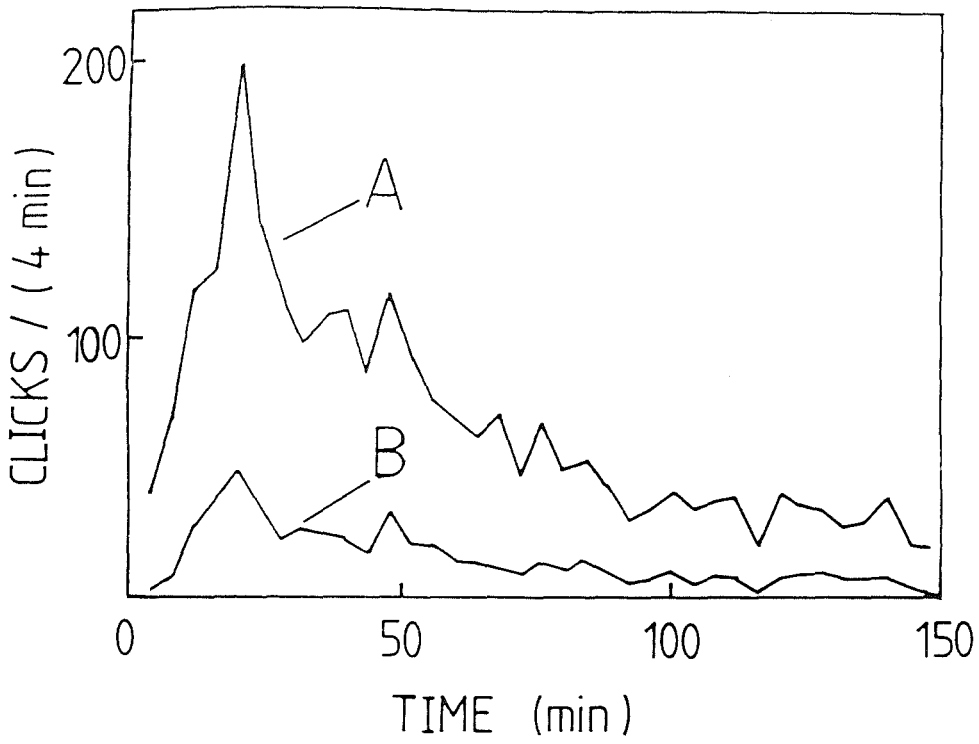


Figure 9. Clicks detected at different discriminator thresholds. The output of a single transducer in contact with the petiolar xylem of a Rhododendron leaf was taken to both channels of the acoustic detector. The discriminator of one channel was put at a low threshold setting (A) and the other at a high threshold setting (B).

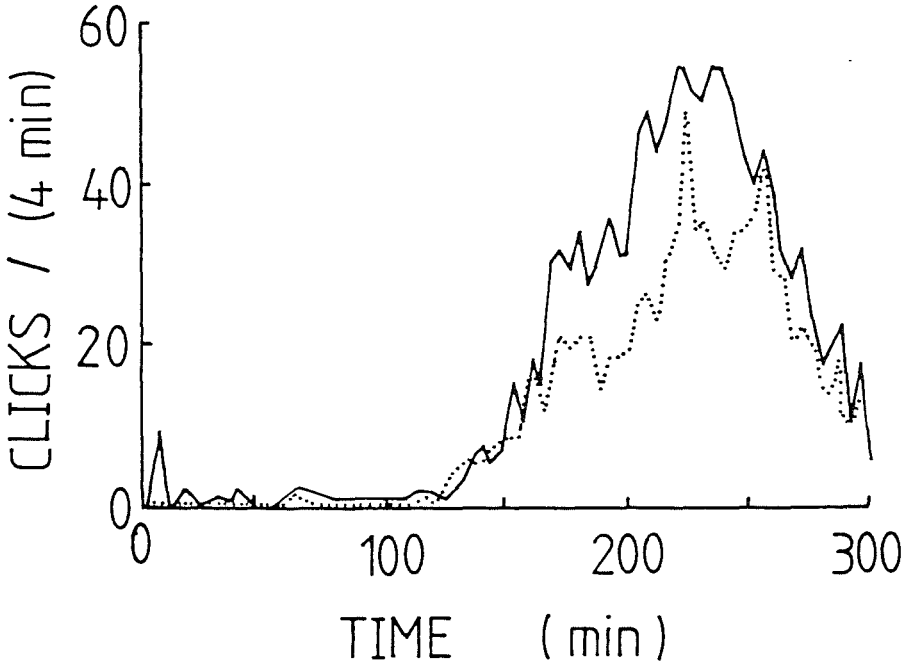


Figure 10. Clicks detected at different points in a Rhododendron shoot. One detector (———) was mounted near the cut end of a 200mm Rhododendron shoot, the other (·····) in the petiole of a leaf about 100mm away from the first.

one mounted in the petiole of a leaf in the terminal cluster, the other into the xylem of the stem near its cut end. Clicks detected at each site were recorded on a twin channel chart recorder.

The experiment was repeated seven times with similar results in each case.

The patterns of click frequency against time were the same for detectors inserted at both sites. This was despite the number of clicks detected at one point being up to 3-4 times that detected by the other detector over the same interval on some occasions.

Clicks detected by one of the detectors often corresponded to clicks detected by the other, indicating transmission of the click vibrations over substantial distances. However, many of the clicks were unique to one or the other of the detectors irrespective of which detector recorded the most clicks during the course of the experiment.

In general many more clicks were recorded by a detector inserted into the stem of the shoot than into a petiole on the same shoot. It could not be determined if this was due to better conduction of vibrations in the more solid xylem of the stem than of the petiole or because of a greater number of cavitation events occurring in the stem.

3.2.6. The role of temperature during acoustic experiments

Figure 11 shows cavitation profiles (section 3.5) drawn from the results of acoustic experiments conducted with or without prior warming of the sound-proofed box in which the experiments were conducted.

These experiments were conducted to test whether differences in transpiration rates between experiments conducted at different temperatures might affect the results obtained.

There was no observable difference in the cavitation profiles drawn using data from acoustic experiments conducted in 'cold' (cabinet temperature 293-298K) or 'hot' (cabinet temperature 307-309K) conditions.

In addition, it was feared that leaf temperatures high enough to cause leaf

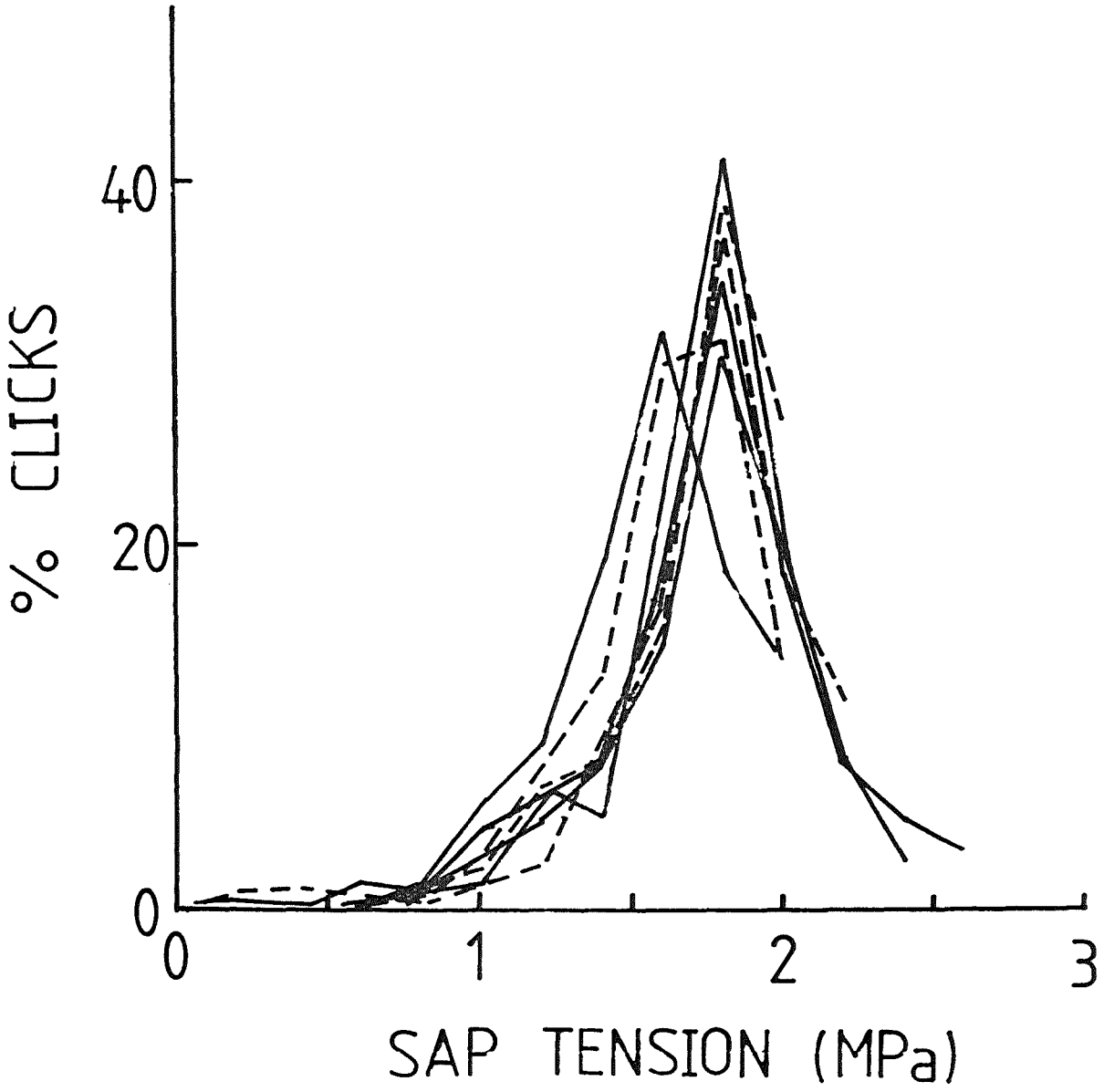


Figure 11. The effect of temperature on determinations of the sap tensions causing cavitation. The cavitation profiles (section 3.5) were drawn up using data from acoustic experiments using Rhododendron leaves and starting when the acoustic cabinet was at room temperature (293-298 K) (———) or had been warmed for half an hour and the inside temperature was about 308K (-----).

damage might result from the use of an incandescent lamp to stimulate transpiration.

Leaf and air temperatures during acoustic experiments were measured using thermocouples soldered from 0.024mm chromel and constantan wire. The temperature of a Rhododendron leaf mounted on the acoustic detector was monitored by a thermocouple held against the side away from the incandescent lamp speeding transpiration. A second thermocouple positioned in the air several centimetres away and shielded from the lamp by a square of aluminium foil measured air temperature.

The results showed that during an acoustic experiment leaf and air temperatures rose to 309.5 K and 308 K respectively over the first hour, and remained at these levels for the remainder of the experiment. When the cabinet door was opened, air temperature fell rapidly to ambient but leaf temperature only fell by about 0.5K, suggesting that leaf temperature was conditioned mainly by radiation load from the lamp, as suggested by Milburn (1973a).

As leaf temperature remained within reasonable limits, no efforts were made to control either leaf or cabinet air temperature during acoustic experiments. However, the lamp used to speed transpiration by the leaf was usually switched on half-an-hour or more before starting experiments so that the balance on top of the sound-proofed cabinet would not suffer changes in tare readings as it warmed up during experiments.

3.2.7. Discussion of sections 3.2 - 3.6

The acoustic technique. Before using the acoustic technique to investigate the sap tension at which cavitation occurs, it was necessary to know the limitations in the technique itself. The results which have been presented confirm those obtained by earlier workers (Milburn and Johnson, 1966; Milburn, 1973 a and b; Milburn and McLaughlin, 1974; and Nonhebel, unpub.) who used a variety of material (Ricinus, Plantago and Lycopersicum) for their experiments.

Rhododendron ponticum was used for most of the experiments because it

produced loud clicks with a minimum of accompanying noises (for instance, from rubbing of leaf parts), experiments using it ran over convenient times (two-three hours) and the leaves and stems were mechanically tough.

The pattern of click frequency with time during acoustic experiments was similar to that reported for leaves of other species (see above). Also the rapid onset of a click and of the apparent dependence of the frequency of the detector output signal on the geometry of the leaf and detector combination are similar to the results obtained using Ricinus leaves on a more massive magnetic transducer (Milburn and Johnson, 1966). It was decided that a simple discriminator acting on the amplitude of the detector output would be sufficient for automating the recording of cavitation. The variability in the frequency and duration of transducer signal output for each click was thought to preclude any selection of signals by methods based on their frequency or duration.

The selection of clicks on the basis of loudness compounded the variability in the number of clicks recorded, which was already present due to differences in the contact of the detector needle and the xylem, the weighting of the detector elements and the performance of the detector transducers.

However, the pattern of clicks detected did not seem to depend on the loudness of the clicks selected for recording. This confirms the results obtained using Ricinus (Milburn, 1973b). Because the pattern of clicks generated during acoustic experiments was not dependent on the detector sensitivity (section 3.2.4) or on the placement of the detector in the xylem (section 3.2.5), it was considered that, if the pattern of clicks was used, it was possible to use the acoustic detector to compare cavitation phenomena between leaves of different species.

The rapid response of click frequency to changes in transpiration rate is very similar to that recorded in Ricinus (Milburn, 1973a). This was taken as evidence that sap tensions were likely to be nearly equal in all parts of the xylem of the sample, as was concluded by Milburn (1973a). In addition, the rapid response was also taken to indicate that cell and xylem water potentials were not far from equilibrium. This is important as sap tension in leaves

during acoustic experiments can then be calculated using the relation of sap tension to RWC for similar leaves in which cell and xylem water potentials were in equilibrium (section 3.5).

3.2.8. Observation of gas arising in xylem conduits during acoustic experiments

In figure 12 and plate 3 are presented the results of an experiment in which the appearance of gas in the conduits of an Acer shoot was recorded at the same time that clicks were monitored by the acoustic detector.

The experiment required for its execution plant stems in which the xylem was sufficiently translucent that bubbles in individual conduits could be observed. The xylem must also be able to be exposed with a minimum of manipulation and damage to the stem. It was advantageous if the material produced loud clicks and could be quickly stressed to cavitating sap tensions.

Branches of Acer pseudoplatanus met all these requirements.

Bubbles could also be seen in the xylem of Pelargonium stems. However, this species was unsuitable for this experiment as (a) the clicks produced were very quiet and (b) the rate of water loss under moderate conditions was so slow that over the long periods necessary for experiment the exposed xylem became opaque.

(a) Clicks and the appearance of gas in xylem conduits

Figure 12 and plate 3 show the results of an experiment conducted using an Acer shoot. In June 1982 branches 1-1.5 m in length were cut underwater from 8-9 year old Acer plants growing in the grounds of the laboratory. The branches were hydrated (section 2.1.1) overnight before experiments. Before experiments began the shoots were trimmed to leave 100-150 mm of the previous year's shoot below the node between that and the current season's growth.

Two branches were held against a stiff board by adhesive tape and 40mm of xylem of the current season's shoot growth exposed by peeling back the bark.

One shoot was set to transpire Indian ink suspension (section 2.7), and the

other distilled water from .02 dm^3 phials. Care was taken that at no time were the ends of the shoots exposed to air.

One channel of the twin channel acoustic detector was connected to the xylem of each shoot by inserting the detector needle into the stem about 40mm above the exposed part of the xylem. By inserting the needles from the side it was possible to mount the detectors without damaging the xylem strands exposed lower on the stems.

A photographic lamp speeded transpiration by the shoots and provided light for photography.

Photographs were taken at intervals (indicated in figure 12) and click production by each shoot monitored continuously.

Unfortunately the background noise levels were very high because the experiments had to be conducted outside the sound-proofed box. Consequently, discriminator thresholds were set at high levels and detected click frequencies were correspondingly low. Also, the detector channel monitoring the control shoot (transpiring distilled water) was functional for only part of the experiment.

Because of these problems the results shown in figure 12 are not as clear as they might be. An indication of the high noise levels during the experiment is seen from the apparent synchrony between patterns of clicks detected in the control and dehydrating shoots (figure 12). Aural monitoring of both detector channels at intervals confirmed that many 'click'-like noises were heard on the channel monitoring the dehydrating shoot but were almost entirely absent from the control shoot. Very high levels of rubbing noises could be heard on both channels as the shoots moved slightly against the backing board.

The increase in the contrast of the vascular strands against the green ground tissue of the stems is easily seen as the experiment progresses (plate 3). At the same time there is an increase in the number of noises recorded by the acoustic detector. The first appearance of gas between parts B and C of plate 3 occurs at the same time as click frequency first starts to rise (figure 12). The increasing contrast between the vascular strands of the stems and the ground

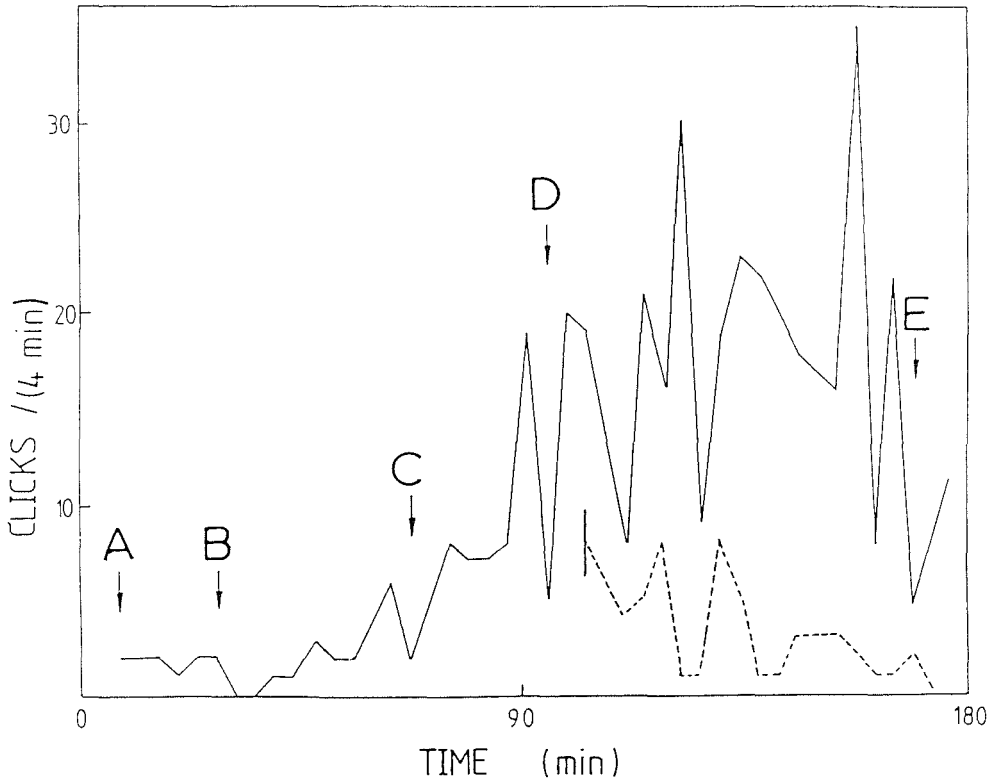


Figure 12. Click frequency in Acer shoots transpiring Indian ink (———) or distilled water (-----). Part of the record of clicks in the shoot transpiring distilled water is missing because of an equipment fault. The arrows indicate the times at which the photographs of plate 2 were taken.

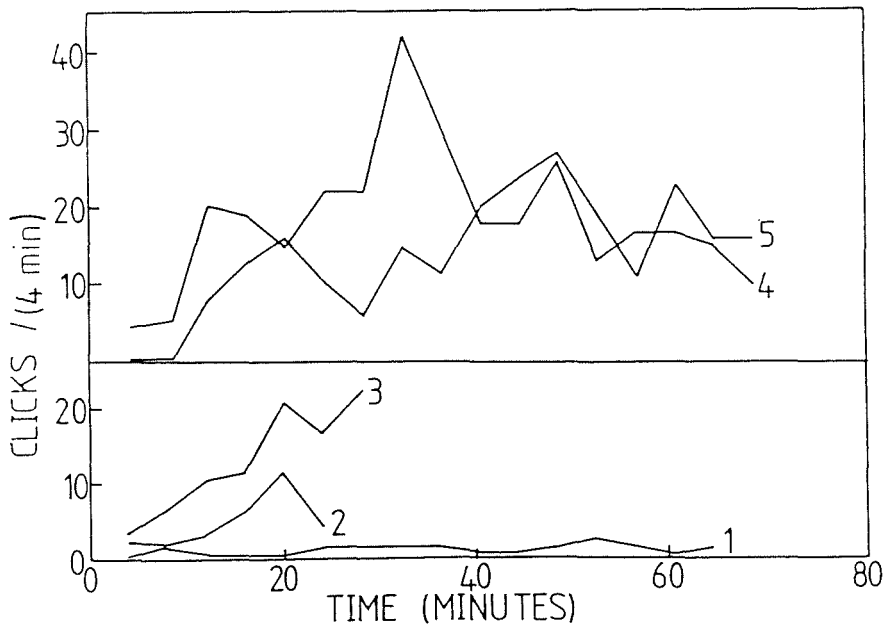
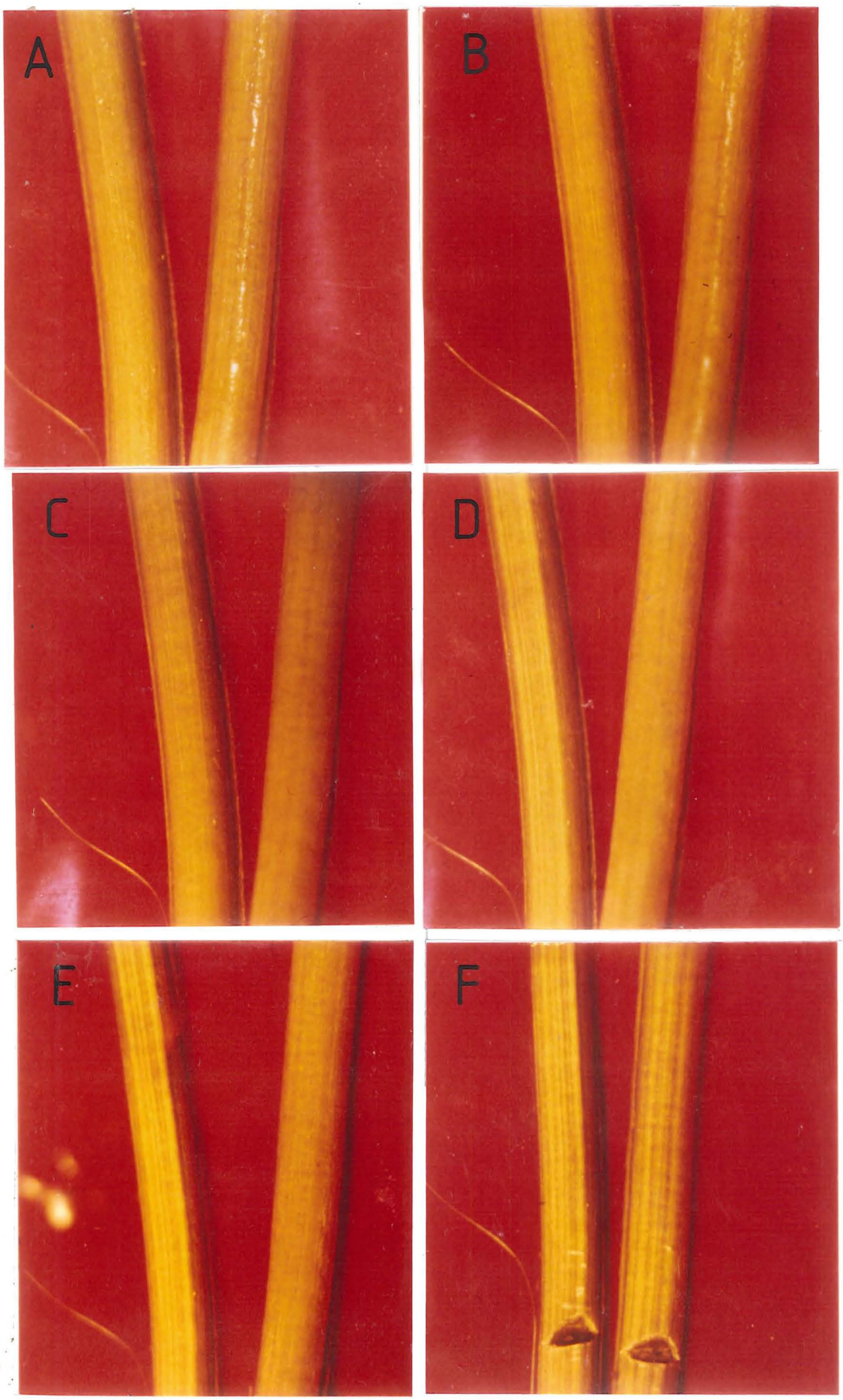


Figure 13. Click frequency in Acer leaves until removed from the acoustic detector. Balance pressures of leaves removed from acoustic detector when click frequency was:

a) rising	b) near maximal	c) declining
Leaf 1 - 0.35 MPa	Leaf 3 - 1.13 MPa	Leaf 5 - 0.90 MPa
Leaf 2 - 1.03 MPa	Leaf 4 - 1.37 MPa	

Plate 3. The appearance of gas in the vascular bundles of Acer shoots when water stressed. The shoot of the left is transpiring Indian ink, that on the right distilled water. The photographs were taken at the times indicated in figure 12. At the end of the experiment air was admitted into the vascular strands of both stems by cutting notches in the stem.

The presence of gas in the xylem conduits is indicated by a lighter colour of vascular strands. This is first seen in the shoot transpiring Indian Ink in part C and develops progressively in D and E. No gas is seen in the xylem of the shoot transpiring distilled water until air was deliberately admitted into the conduits by notching the xylem (part F). (x 2)


A
B
C
D
E
F

tissue seen in parts D-E of plate 3 is concurrent with a continuing high click rate. At the end of the experiment the contrast between the vascular strands and the ground tissue in water stressed stems is seen to be very similar to that occurring when air is admitted to the xylem of a previously unstressed stem (plate 3, part F). The similarity of appearance between conduits in stems which have been cavitated by imposition of high sap tension and those to which air has been admitted strongly suggests that the increasing contrast is due to the appearance of a gas phase in the xylem.

At the end of the experiment the shoot transpiring Indian ink suspension was severely wilted and a leaf removed from it found to have a balance pressure of 1.10 MPa. The shoot transpiring distilled water was still unwilted and a leaf removed from it had a balance pressure too low to be accurately assessed by the gauge on the pressure chamber (i.e balance pressure was less than 0.05 MPa).

(b) Testing for entry of air into xylem conduits

Two Acer shoots similar to those used above were retrimmed under water so that only 45mm of stem remained below the node separating the current and previous seasons' growth. The shoots were then set to transpire the Indian ink suspension (section 2.9) until the leaves had dried to brittleness (this occurred if the shoot was set to transpire the ink suspension on a laboratory bench overnight).

When these stems were sectioned ink was seen to occupy the vessels of both the current and the previous seasons' xylem in a section cut approximately 15mm below the node separating the current and previous years' extension growth. In another section cut 15mm above the node no ink was found.

These results were taken as evidence that the apical movement of ink in the transpiration stream was halted in the region of the node. This was presumed to be due to the presence of pit membranes in the node.

Also, as the leaves of the stems dried to brittleness (which did not happen at leaf balance pressures between 0 and 1.37 MPa), without ink being drawn through

these pit membranes, the experiment was taken as indicating that the pit membranes did not rupture when subjected to differential pressures of as much as 1.37 MPa. If the membranes had ruptured ink would have passed to the vessels in the xylem of the current year's shoot. This did not happen in this experiment or in the acoustic experiment described previously.

(c) The balance pressure of cavitating Acer leaves

Figure 13 shows the results of an experiment conducted to determine the approximate sap tensions at which clicks occurred in leaves from shoots similar to those used in the preceding experiment.

Acer leaves from shoots similar to those used in the preceding experiments were mounted separately on the acoustic detector and click production monitored. Leaves were removed from the acoustic detector and their balance pressures determined when (a) clicks were just beginning, (b) were occurring near the maximal frequency and (c) click frequency had begun to decline after the maximum.

The balance pressures of the leaves for which click production was monitored are given in the legend to figure 13.

Clicks were first heard in leaves when balance pressures were slightly in excess of 1 MPa and had started to fall by the time balance pressure had reached 1.3 MPa.

3.2.9. Discussion

The evidence linking acoustically detected clicks to cavitation of xylem sap is mainly circumstantial. Dehiscence of fern sporangia was reported to be accompanied by a 'click' (Milburn and Johnson, 1966). The click was presumed to be due to cavitation of sap in the cells of the sporangial wall although it may also have been caused by the elastic recoil of the opening sporangium.

An audible click was associated with the fracture of water in sealed glass

tubes (Dixon, 1914) but the direct linking of clicks (detected by the acoustic detector) to cavitation of xylem sap has not yet been achieved. In the experiments cited by Crafts et al., 1949) there was no record of audible clicks occurring in conjunction with the appearance of gas in the xylem.

Milburn and Johnson (1966) linked acoustically detected clicks to cavitation of xylem sap on the basis of circumstantial evidence, including a decrease in the rate of water uptake by Ricinus leaves stressed to the same extent as those in which clicks occurred (Milburn, 1966). Later work (Milburn, 1973 a and b; Milburn and McLaughlin, 1974) showed that (a) clicks were apparently not all due to tissue fracture, (b) their frequency could be altered by changing the rate at which sap tension changed, (c) the recovery of clicks in previously dried material was not immediate and was not dependent on whether non-xylem tissues were alive or dead.

The results of experiments with Acer (section 3.2.8) have shown that gas appeared in the vascular strands of young shoots at the same time that clicks were detected in the shoot. It was unlikely that the gas could have entered from the cut end of the shoots as (a) the cut end was submerged under the surface of a liquid (diluted Indian ink) and (b) a similar shoot transpiring distilled water maintained its water potential, indicating that the vessels of the basal end of the shoot were still able to conduct water and were not embolised by gas admitted to the conduits during preparation for the experiment. Also, as Indian ink did not appear in the vascular strands of the shoot, it was unlikely that the pit membranes of the node between the current and previous years' shoots had ruptured to admit air to the parts of the xylem under observation.

Experiments also showed that clicks were detected in the Acer shoot and in leaves detached from similar shoots at sap tensions of between 0.9 and 1.2 MPa. This is slightly lower than the sap tensions at which cavitation was expected in Acer (table 8). However, cavitation was found to occur at lower sap tensions in immature than in mature Rhododendron leaves (table 9). As the Acer samples used in the above experiment were not yet fully mature (they

were sampled early in the summer) the slightly lower sap tensions at which cavitation occurs in these experiments may have been a reflection of this immaturity.

Although gas appeared in the xylem at the same time as clicks were detected, and at a time when sap tensions were comparable to those at which clicks were detected in leaves, it was not possible to link the appearance of gas in individual conduits to a particular 'click' for two main reasons. Firstly, the xylem in each strand consisted of considerable numbers of vessels overlying each other and also overlaid by fibres and parenchuma. It was therefore unlikely that one should be watching, or be able to see, a particular conduit at the time that it cavitated.

Secondly, the acoustic detector had been mounted so that its needle was inserted into different vascular strands than those which were exposed for observation. Although the vibrations produced by cavitation in a conduit in one strand may have been detectable by the acoustic detector mounted in another (section 3.2.5), they would be expected to be very much fainter than those occurring in the strand into which the detector had been mounted and may not have been passed by the discriminator.

Although the experiments linking clicks to the appearance of gas in Acer xylem provide more direct evidence that clicks are the result of sap cavitation than do the experiments conducted by Milburn (see above), the direct linking of a click to a single observed cavitation event has not yet been achieved.. In addition, although the experiments with Indian ink indicate that the gas observed in the xylem did not enter from the cut ends of the conduits, the experiment did not help to resolve whether the gas entered the xylem from elsewhere, for instance from gas filled fibres or wounds, or arose entirely within the conduit.

3.3. Determination of sap tension in leaves during acoustic experiments

3.3.1. Introduction

Sap tension in samples on the acoustic detector can be estimated in several ways. These may involve measurement of sap tension of the leaves at intervals during acoustic experiments, the rehydration of leaves from acoustic experiments and measurement of balance pressure during a subsequent drying cycle, or the measurement of sap tension and clicks in different groups of leaves.

Sap tensions can be found from balance pressure measurements if certain conditions are met. The most important condition is that the distribution of water in the leaf is the same when the leaf is held at the balance pressure as it was before excision from the plant. This relation may be altered by entry of air into the xylem conduits as the leaf is dried on the laboratory bench or by the evacuation of water from xylem conduits after cavitation. Refilling of conduits emptied by either process may cause over-estimation of the turgor component (and therefore sap tension) of the sap in the remaining water filled conduits. This problem is investigated in the next section (3.4.2).

The most direct way of measuring sap tension during acoustic experiments would be to measure the water potential of the leaf on the acoustic detector psychrometrically as the experiment progresses. Sap tension can then be calculated from the difference between Ψ_l and the osmotic potential (Ψ_s) of the sap (Boyer, 1969).

In practice this method is unsatisfactory for several reasons. It is unlikely that the thermal stability required for the successful use of psychrometric methods will be obtained under the conditions used for acoustic experiments (Campbell and Campbell, 1974). Periodic sampling of leaf discs for measurement of Ψ_l in separate psychrometer chambers causes damage to the leaf and results in uneven drying across the lamina. In addition, considerable

variability in Ψ_l is known to occur across the leaf (Masip, 1979; Campbell et al., 1966) so that the calculated sap tension will be partly dependent on from which part of the leaf the sample was taken.

By contrast, the pressure chamber can be used to obtain averaged sap Ψ_p for the leaf more quickly than can the psychrometric technique.

The pressure chamber can be used to measure sap Ψ_p in leaves removed from the detector during acoustic experiments. However, this approach is limited by the infrequency with which sap Ψ_p can be determined during experiments. In addition, errors in sap pressure determinations may be caused by damage to the leaf xylem caused by mounting on the acoustic detector (Slavik, 1974).

It is therefore desirable that indirect methods be used to assess the sap tensions in leaves during acoustic experiments. This aim was accomplished by drawing up calibration curves of sap Ψ_p against RWC for leaves of each species used in acoustic experiments. As changes in leaf RWC during acoustic experiments were known this calibration could then be used to estimate the sap tensions in these leaves.

The pressure chamber can be used to derive the relation of sap tension to RWC of the leaves from acoustic experiments or from other, similar leaves. Moreover, the relationship can be derived by detailed study of a few leaves or by a less intensive study of many.

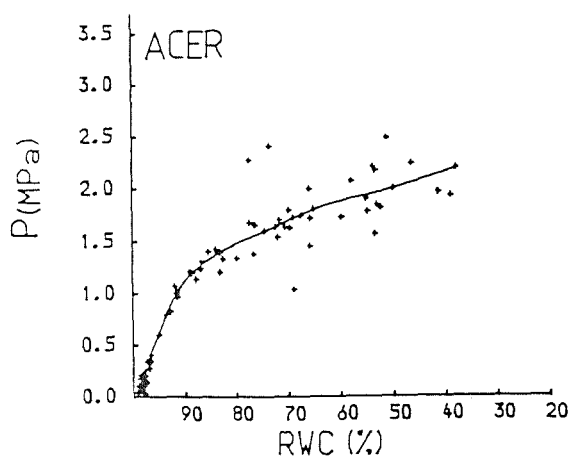
It was decided that the best way of obtaining a useful calibration of sap tension against RWC for this study was to measure at intervals the balance pressures of many leaves as they transpired on a laboratory bench. The reasons for selection of this technique and the rejection of other possible methods are given below.

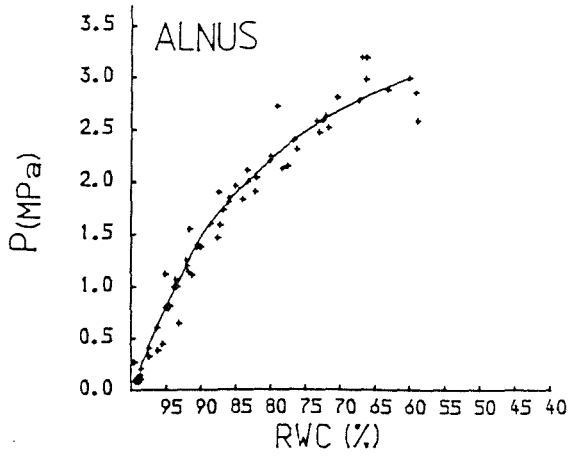
3.3.2. The relation of balance pressure to RWC

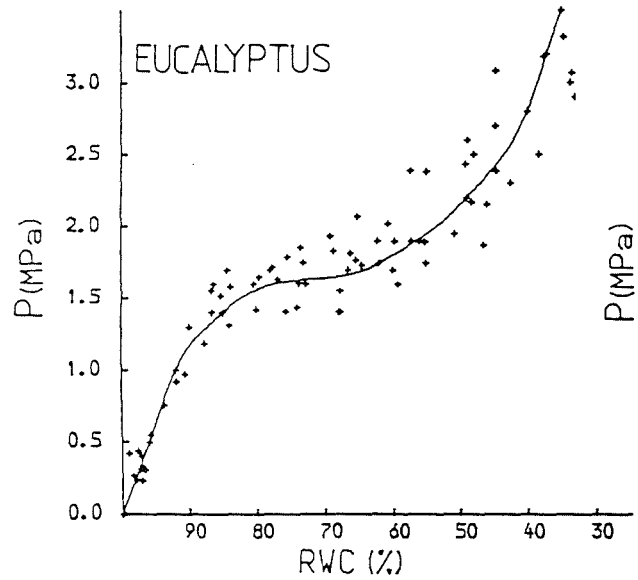
Calibration curves of balance pressure against RWC for leaves of the species used in acoustic experiments are shown in figures 14 and 15.

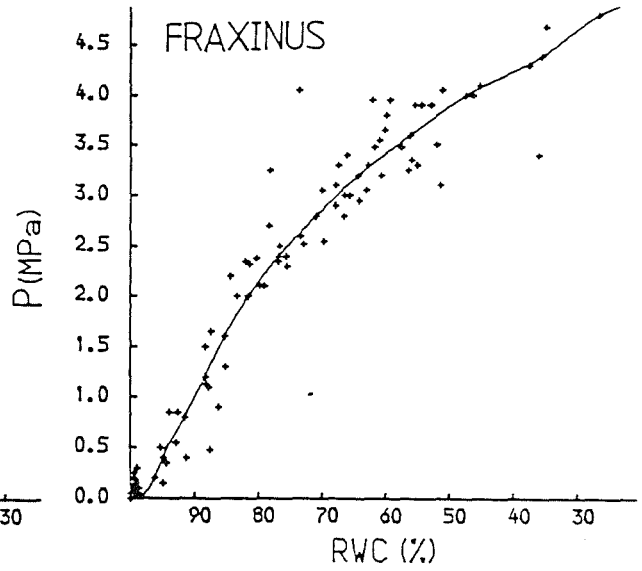
Hydrated leaves similar to those used in acoustic experiments were allowed

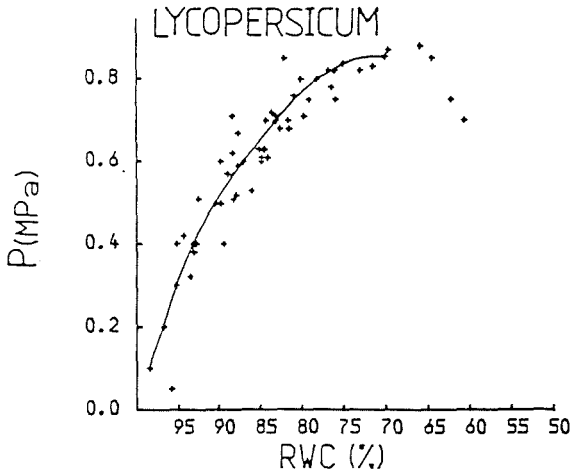
Figure 14. 'Calibration curves' relating balance pressure (P) to RWC for leaves of all species for which cavitation profiles were constructed. In the case of Acer, Plantago and Rhododendron only one example of the relation is shown as the several collections made differed only slightly.

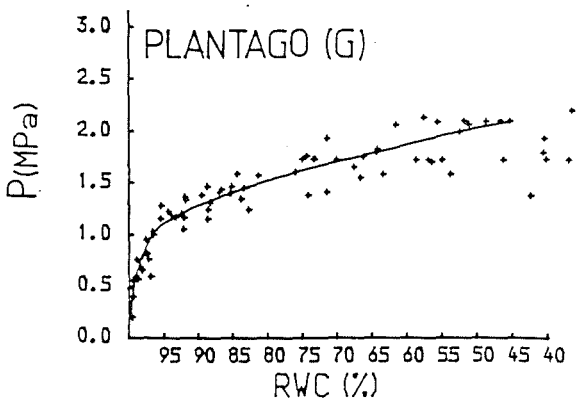

ACER
P(MPa)
RWC (%)

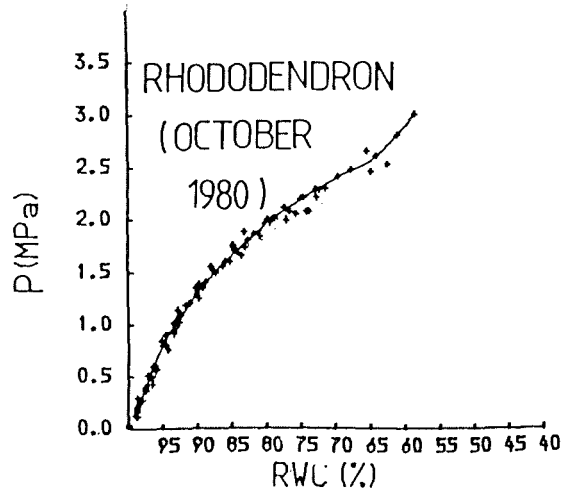

ALNUS
P(MPa)
RWC (%)

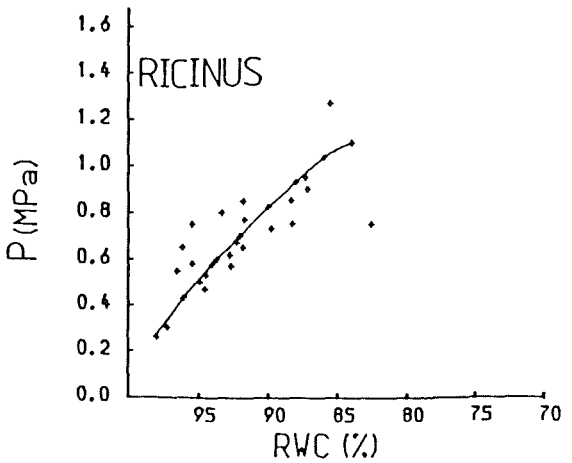

EUCALYPTUS
P(MPa)
RWC (%)


FRAXINUS
P(MPa)
RWC (%)


LYCOPERSICUM
P(MPa)
RWC (%)


PLANTAGO (G)
P(MPa)
RWC (%)


RHODODENDRON
(OCTOBER
1980)
P(MPa)
RWC (%)


RICINUS
P(MPa)
RWC (%)

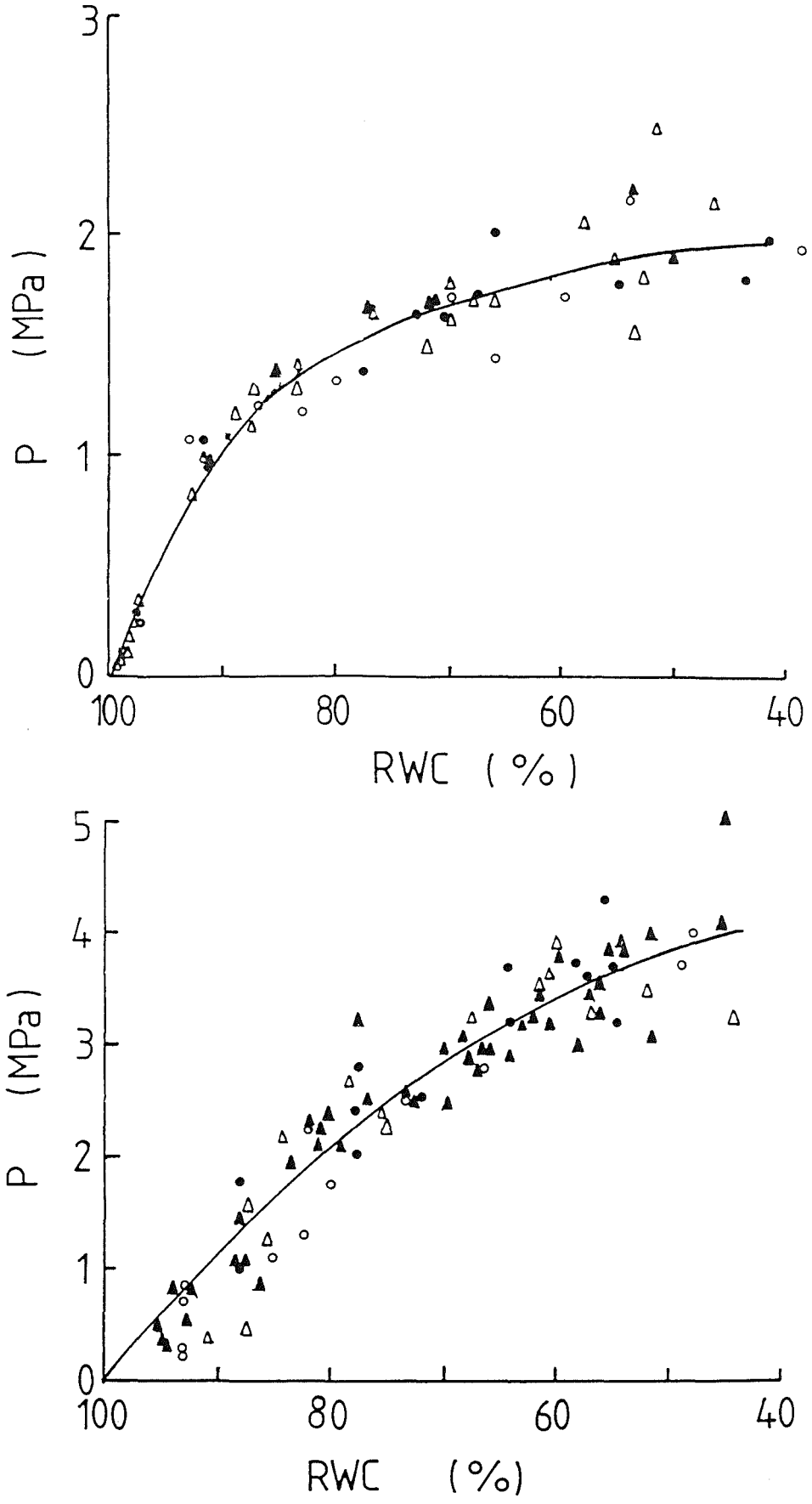


Figure 15. The relation of balance pressure (P) to RWC of a) Acer and b) Fraxinus leaves constructed from experiments using air (o) or nitrogen (Δ) in the pressure chamber. Open symbols indicate a first balance pressure measurement with a particular leaf, closed symbols, subsequent measurements.

to transpire on the laboratory bench and their balance pressures measured at intervals. An exact duplication of drying rates of leaves used in acoustic experiments and those used in drawing up the relation of sap tension to RWC was not attempted. It was considered that (a) sap tensions and Ψ_l were probably close to equilibrium in leaves drying rapidly on the acoustic detector (section 3.2.3) as well as in those drying more slowly on the laboratory bench and that (b) any inequilibria in the relation caused by drying rates would probably disappear in the time taken to make a balance-pressure measurement.

Multiple measurement of balance pressure on each leaf

Several balance pressure measurements were made with each leaf, unless it had been damaged on a previous measurement, in which case the leaf was discarded.

As is seen in figure 15b multiple use of Fraxinus leaves in this manner did not result in an observable change in the relation of balance pressure to RWC from that found when leaves were used only once. This was found to be true for all species except those with very soft petioles (e.g. Lycopersicum and Ricinus) which were almost always damaged by the pressure chamber seal.

Two factors capable of causing changes in the relation of leaf balance pressure and RWC were thought worthy of further investigation. These were

i) temperature changes occurring during pressurisation and depressurisation of the pressure chamber and

ii) narcosis and anaerobis which might occur if the leaf is held at high pressures for long periods in the pressure chamber.

i) Temperature changes in the pressure chamber

Figure 16 shows the result of an experiment conducted to measure the changes in leaf temperature occurring during a typical balance pressure measurement. Chromel-constantan thermocouples were held against a Rhododendron leaf or left free in the air of the pressure chamber. A pressure cycle at rates of pressure increase and decrease similar to those used in measuring balance pressure and extending to a maximum pressure of 4.14 MPa was undertaken.

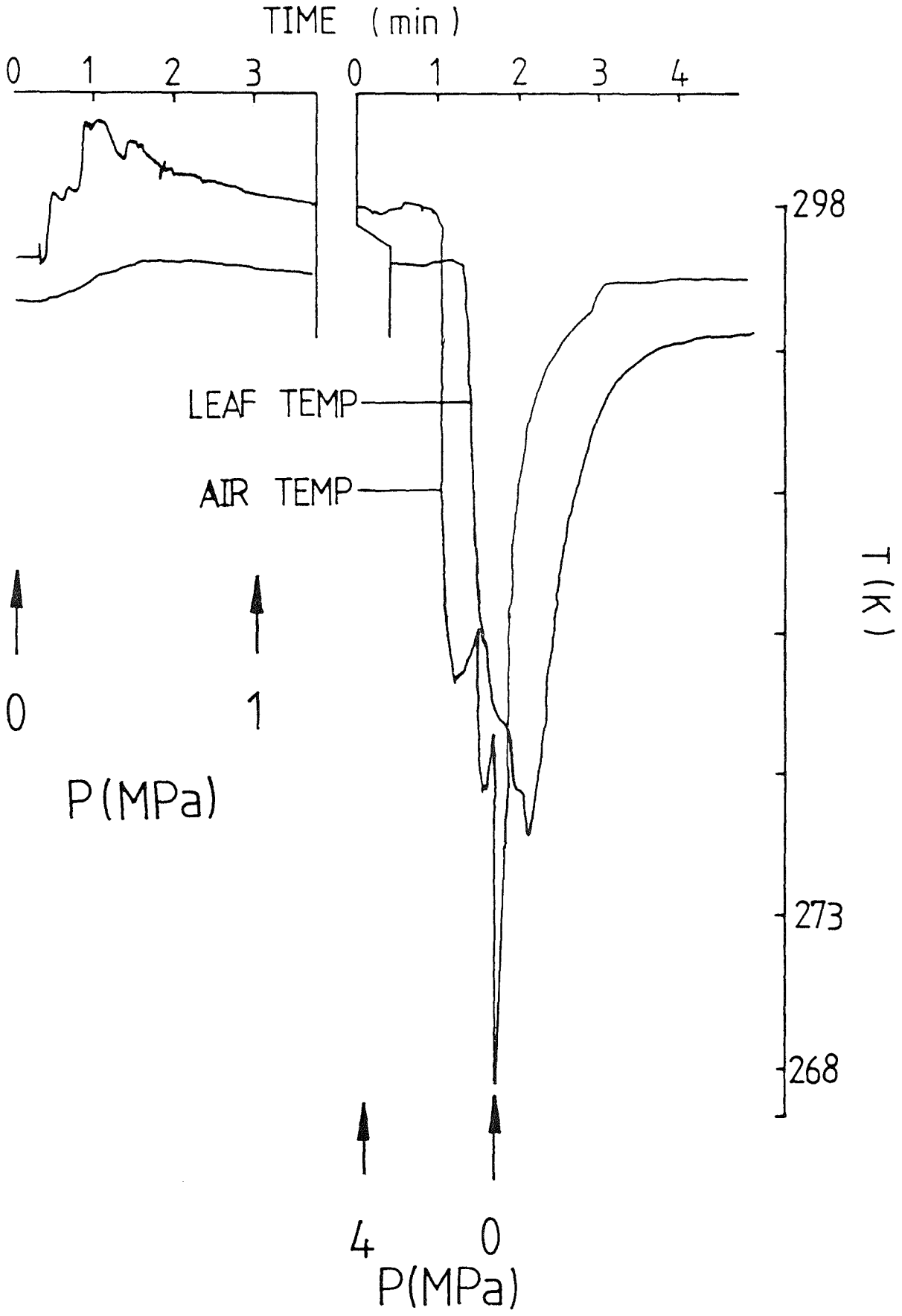


Figure 16. Temperature changes occurring during measurement of balance pressure of a Rhododendron leaf. Only the most extreme changes in temperature are shown. Records of leaf and air temperature when pressure is falling have been separated slightly to improve clarity.

Temperature changes were greatest when the first increments of pressure were applied or the last increments released. Air temperature was more variable than leaf temperature (figure 16). The maximum increase in air temperature was 4.5K whereas the maximum for the leaf was 1.3K. When pressure was released both leaf and air temperatures fell markedly, the leaf to 275.4K, the air to 266.5K. The leaf returned to room temperature within 3 minutes if left in the pressure chamber and within a minute if removed from the chamber immediately that pressure was released, the usual procedure when constructing the calibration curves.

There did not appear to be discernible adverse effects caused by multiple determinations of balance pressure on a single leaf (figure 15). Temperature measurements during a typical pressure cycle indicated that, as long as the rate of pressure increase was slow, temperature increases during an experiment were of only a few degrees (figure 16). Temperature changes during pressure release were much greater with chamber air temperature falling to well below freezing. Leaf temperature fell less, probably due to the thermal mass of the leaf (Puritch and Turner, 1973) and did not fall below freezing.

These temperature changes were short lived and apparently did not affect the relationship of balance pressure to RWC.

ii) Effects of exposure to gas at high pressure

Figure 17 shows the effect on leaf balance pressure of prolonged exposure of leaves to air at high pressure. Calibrations of balance pressure against RWC drawn up using air or nitrogen are shown in figure 15.

Leaves of *Acer*, *Fraxinus* and *Rhododendron* were exposed on the laboratory bench to develop water deficits and then mounted in the pressure chamber. Evaporation from the leaves whilst in the pressure chamber was minimised both by enclosing the leaf in a small, prehumidified plastic bag, and by lining the pressure chamber with moist paper towel (Jones and Higgs, 1980; Tyree *et al*, 1978).

The chamber was pressurised with air and the balance pressure determined. The pressure was then released by 0.1-0.2 MPa and maintained at this level.

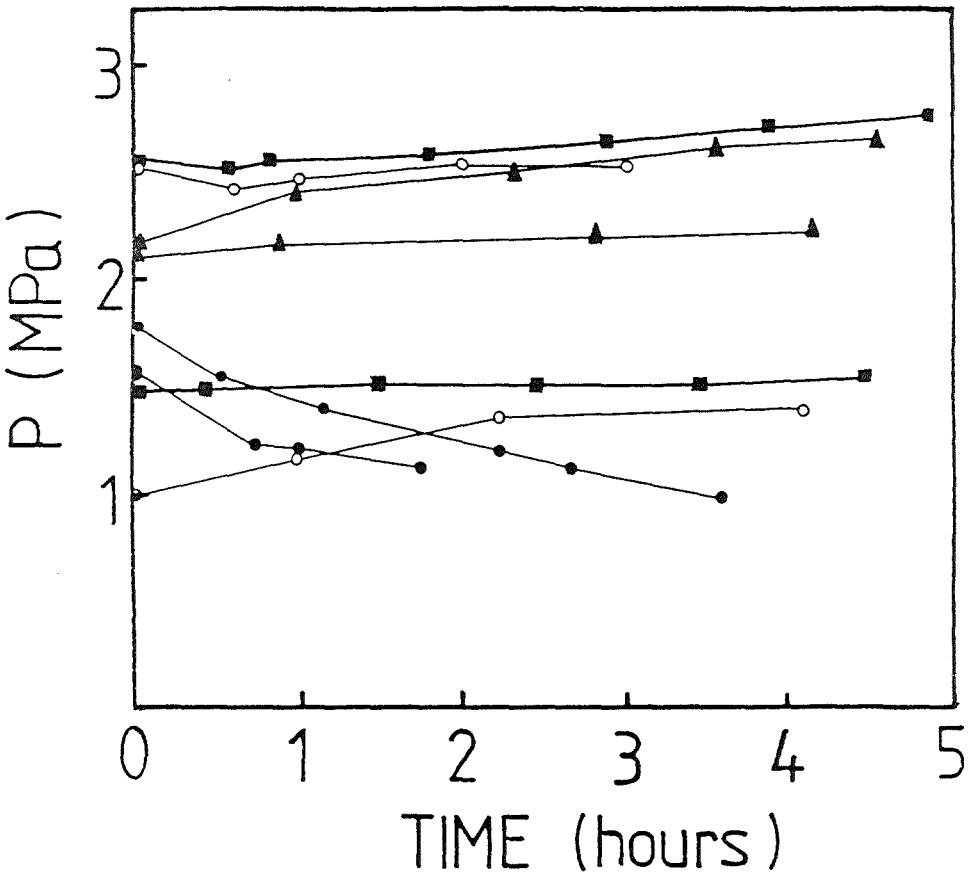


Figure 17. The effect on leaf balance pressures of long periods of exposure to high pressures of air (closed symbols) or nitrogen (open symbols).

o Acer

Δ Fraxinus

□ Rhododendron

Leaf balance pressures were determined at intervals over the duration of the experiment.

The balance pressures of Fraxinus and Rhododendron rose slowly during the period of the experiment. This rise was attributed to slow evaporation from the leaves in the pressure chamber. This was probably partly due to leaks of gas from the pressure chamber occurring through the petioles of the leaves and from around the pressure chamber seals. Weight losses from leaves in the pressure chamber are given in table 2.

Table 2. Weight losses from leaves during long periods in the pressure chamber.

Species	Weight lost as % leaf fresh weight (± SE)
Acer	3.3 ± 0.8 (n = 4)
Fraxinus	0.6 ± 0.5 (n = 2)
Rhododendron	1.4 ± 0.2 (n = 2)

By contrast balance pressures of Acer leaves subjected to high air pressure for long periods were found to decrease. The same leaves were found to have brownish patches between the major veins and to have a bad smell when removed from the pressure chamber. These symptoms were not found with Fraxinus or Rhododendron leaves after pressurising with air.

As Acer leaves appeared to be the most sensitive to air at high pressure leaves of this species were then used in similar experiments in which oxygen free nitrogen was used to pressurise the chamber. The results are included in figure 17. When nitrogen was used the balance pressures of Acer leaves were found to increase very slightly with time. When removed from the pressure chamber the leaves were not discoloured and did not have a bad smell.

As nitrogen appeared to be less damaging to leaves of a sensitive species

such as Acer than was air, nitrogen was used for most balance measurements made during this project and for all studies requiring that the samples be held at pressure for long periods.

Short-term effects of gas on balance pressure measurements

Comparisons of balance pressure measurements made using air or nitrogen were undertaken using leaves of Acer, Fraxinus, Plantago and Rhododendron. There did not appear to be any differences in the relation of balance pressure to RWC arising from the use of air or nitrogen in the pressure chamber.

Therefore it was considered that calibrations of leaf balance pressures against RWC drawn up using air in the early part of the project could be safely used in analysis of the relation of sap tension to cavitation (section 3.5) and compared with those drawn up later when nitrogen was used in the chamber.

3.3.3. Other methods of obtaining the relationship between balance pressure and RWC

Attempts were made to obtain the relation of leaf balance pressure to RWC by constructing pressure-volume (PV) curves (Scholander et al., 1964) and by rehydrating leaves from acoustic experiments. These techniques were subject to considerable experimental errors (discussed below) which could be avoided by intermittent measurement of balance pressure of similar leaves drying on the laboratory bench. Also, these techniques were generally concerned with obtaining a detailed relation of balance pressure to RWC in a single leaf. Their application to a sufficient number of leaves to show the range of variability within material would be extremely tedious.

i) Construction of P-V curves

The relation of balance pressure to RWC can be obtained by forcing the leaf to lose water by applying overpressure in the pressure chamber. This method also allows more detailed curves to be constructed than the less frequent

measurements of balance pressure of leaves drying on the laboratory bench allow.

P-V curves were constructed for Rhododendron leaves by the procedure of Tyree et al. (1978). Expressed sap was collected in small plastic phials stuffed with paper tissue which was capped by a larger phial immediately after termination of the sap collections.

It was found that evaporation (section 4.5, table 15) from leaves in the pressure chamber and from the collection phials precluded the use of this technique for accurately deriving the relation of balance pressure to RWC at Ψ_1 between full turgor and the point of incipient plasmolysis (section 3.4.4). In addition, the volumes of sap expressed over pressure increments of 0.18 MPa were small (table 15) and evaporation errors were compounded by balance errors.

Construction of P-V curves as a means of obtaining the relation of sap tension to RWC was therefore rejected because of the problem of evaporative water loss. Moreover, the time necessary to derive the relationship in the number of leaves necessary to know the degree of variability between leaves would limit that which could be devoted to other work.

ii) Rehydration of leaves from acoustic experiments

In figure 18 are shown the results of experiments designed to test the feasibility of rehydrating leaves from acoustic experiments for subsequent determination of the relation of balance pressure to RWC.

Acer and Fraxinus leaves were dehydrated on the laboratory bench to the balance pressures indicated in the legend of figure 18. 10mm was then trimmed under water from the petioles to remove gas emboli and the leaves stood with their petioles in phials of distilled water in a humid atmosphere (section 2.1) to rehydrate. At intervals the leaves were removed from rehydration, blotted dry and weighed before returning for further rehydration. At the end of the experiments the leaves were dried and their water contents (expressed as percentages of their water content before stressing) calculated.

Acer leaves quickly regained their lost water content and thereafter

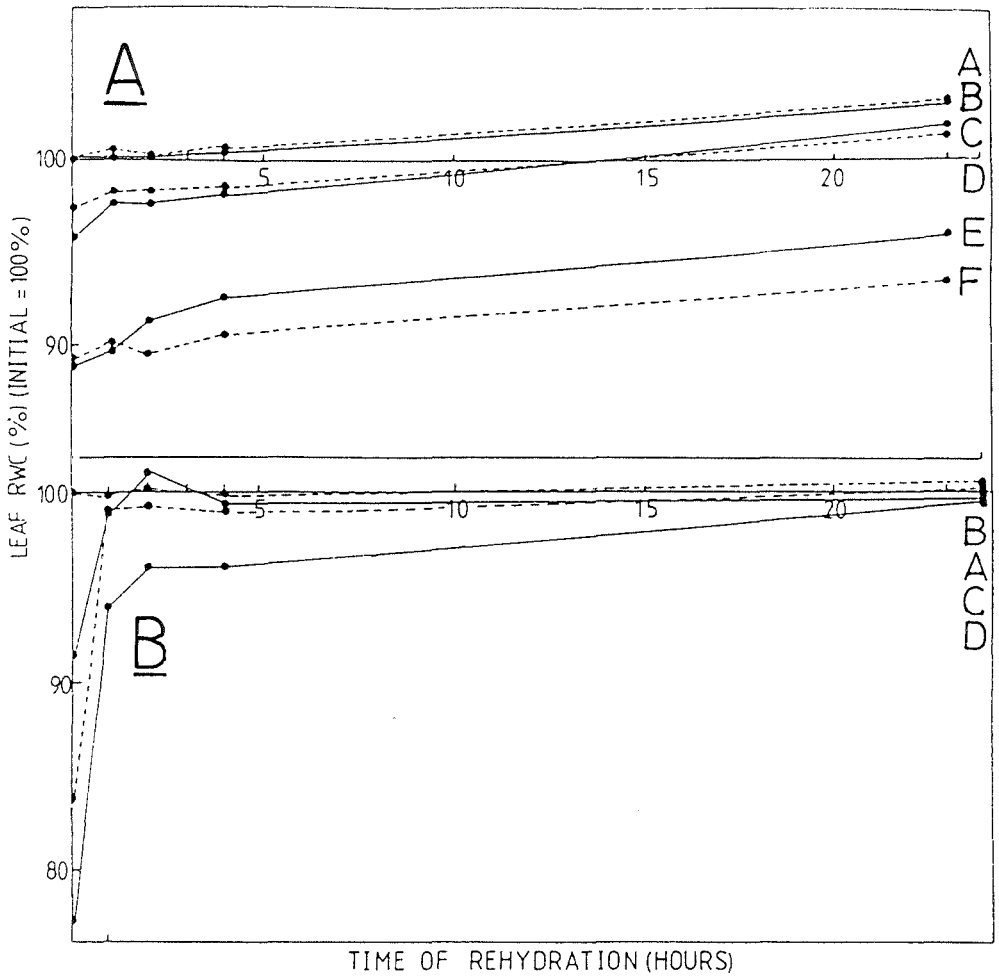


Figure 18. Rehydration of leaves after water stress. Water content during rehydration was calculated using the water content before stressing as 100%.

Leaf balance pressures before rehydration:

A. Fraxinus: A 0 MPa
B 0 "
C 0.32 MPa
D 0.28 "
E 2.50 "
F 1.72 "

B. Acer: A 1.37 MPa
B 0.0 "
C 1.19 "
D 1.36 "

remained at, or very close to, their weights before being stressed.

Fraxinus leaves were slower to rehydrate and severely stressed leaves had not recovered their initial weights after 24-25 hours of rehydration. In addition, lightly and unstressed Fraxinus leaves took up more water than had been lost during the rehydration period.

Rehydration of leaves was therefore rejected as a means of estimating sap tensions in those leaves during acoustic experiments as it was evident from even a small number of trials that the time taken for leaves to recover lost water varied greatly between species and with the magnitude of the original stress imposed. Also, whereas some species (Acer in the experiment) recovered only the water lost during drying others (Fraxinus) regained more than was lost, possibly indicating growth (Milburn and Weatherly, 1971). Although not investigated in these species, it was possible that even in those leaves which recovered only the water lost during stress, the relation of balance pressure to RWC may have altered (for instance due to osmotic adjustment in response to the original stress) between the first and second drying cycle. Because in many species cavitation in some species started while cells retained some turgor (section 3.5) even small differences in the recovery of leaves from water deficits may cause relatively large errors in calculation of sap tensions in cavitating leaves.

In addition, errors in balance pressure determination may result from the damage to the xylem caused by the needle of the acoustic detector (Slavik, 1974; Nonhebel, pers. comm.).

3.3.4. Discussion

Calibrations of leaf balance pressure against RWC were constructed using leaves dried after excision for all the species for which the relationship between cavitation and sap tension was to be determined. In every case it was possible to draw a calibration line through the data. Scatter around the calibration line was moderate and for most species a line could be drawn such

that almost all data points fell within ± 0.2 MPa of it (e.g. figure 15). Variability tended to increase as balance pressures increased. This was partly due to difficulty in obtaining accurate balance pressure measurements in severely water stressed leaves, but may have been a reflection of differences in the desorption isothermsof individual leaves at low Ψ_l.

It was considered that the calibrations of leaf balance pressure against RWC were not likely to be in error due to artifacts arising from either repeated determination of balance pressures using the same leaf or the use of either air of nitrogen to pressurise the chamber. It was apparent, however, that considerable care had to be taken to minimise mechanical damage to leaves in the pressure chamber and also to restrict the use of air to experiments in which the leaf was held at high pressure for only short periods.

Other methods of obtaining the relation of sap tension to leaf water content were rejected as they were found to be subject to greater possible error than was determination of the relationship by using a number of leaves stressed after excision.

3.4. The effect of cavitation on pressure chamber measurements of sap tension and leaf water potential

3.4.1. Introduction

The use of the pressure chamber to measure sap tension and, by allowing for the osmotic potential (Ψ_s) of the xylem sap, leaf water potential (Ψ_l) is based on several assumptions.

Firstly, it is assumed that xylem and cell water potentials equilibrate rapidly. It is probable that this is the case in small samples such as leaves (Scholander, 1964) although quite long periods may be required before equilibrium is attained in larger samples, particularly if they have been subjected to an initial overpressure (Tyree and Dainty, 1973).

Secondly, and of particular importance to this study, is the assumption

that the distribution of water in a sample held at its balance pressure is the same as in the sample before it was detached from the plant. This will not be so if normally empty voids in the xylem or other tissues become filled with water when balance pressure measurements are made.

For leaves or shoots subjected to water stress after removal from the plant, as in the work reported here, these voids may take four forms. They may be

a) xylem vessels emptied as water retreats from the cut surface until held by the pit membrane (Scholander et al., 1965).

b) spaces in the xylem (e.g. embolised vessels and fibres) or in other tissues (e.g. interstitial spaces in the pith) (Boyer, 1967).

c) water-stressed tissues in the sample (Ritchie and Hinckley, 1971).

d) conduits emptied by sap cavitation.

The investigations reported below were carried out to assess the use of the pressure chamber to measure sap tensions and Ψ_1 in leaves and shoots at cavitating sap tensions.

3.4.2. The effect of cavitation on balance pressure of shoots

The balance pressures of Rhododendron shoots and of leaves from these shoots were compared at sap tensions ranging from near zero to greater than those suspected of causing cavitation (figure 19).

The effect of cavitation on balance pressure measurements was investigated in this manner as the relative xylem volume, and therefore the extra pressure required to fill cavitated conduits before sap will appear at the cut end of the xylem, is greater in shoots than in leaves.

Rhododendron shoots were hydrated overnight, trimmed to a stem length of 200mm and the apical bud removed. This left approximately 120-150 mm of leafless stem with a cluster of mature leaves at one end. The shoots were weighed and left to develop sap tensions by transpiring on the laboratory bench. When the desired degree of stress had been achieved the shoot was sealed into

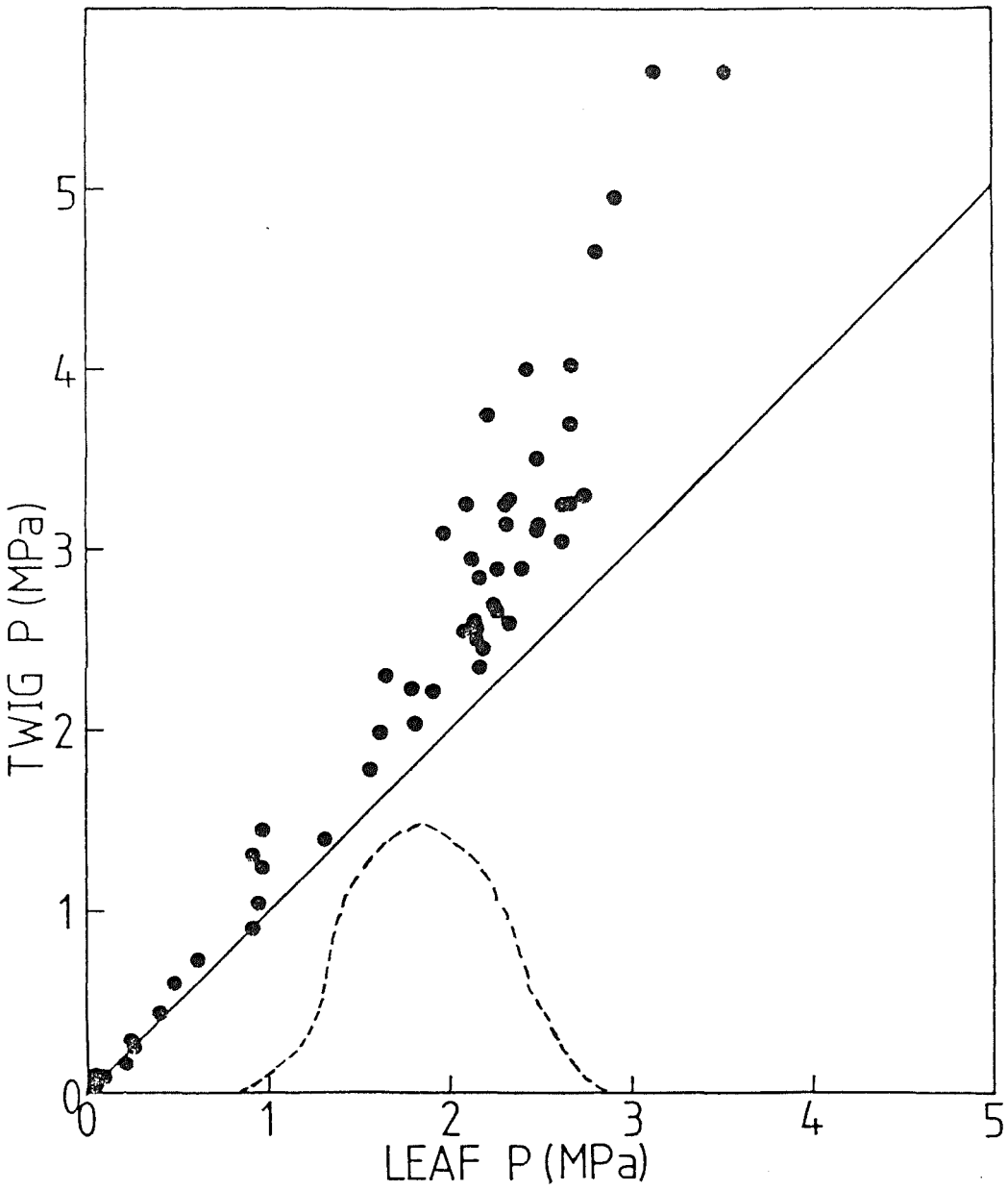


Figure 19. Comparison of balance pressures of Rhododendron shoots with the balance pressures of leaves from the shoots. The continuous line indicates a 1:1 relationship between the two. The broken line indicates the approximate relationship between cavitation and sap tension in Rhododendron leaves.

a plastic bag to equilibrate overnight at room temperature.

After equilibration the shoot was weighed again, one leaf removed and balance pressure of both the leaf and shoot determined simultaneously using two pressure chambers controlled from a single gas admission valve. Leaf and shoot balance pressures are compared in figure 19.

Chamber pressure was increased particularly slowly (at about 0.002 - .003 MPa s^{-1}) to minimise errors due to inequilibrium within the shoot (Tyree and Dainty, 1973).

After measurement of its balance pressure the single leaf was sampled for psychrometric determination of Ψ_l. Sap osmotic potentials were also determined using sap expressed by overpressuring leaves or shoots in the pressure chamber after their balance pressure had been measured.

At balance pressures between 0 and 2 MPa shoot balance pressures were only slightly higher than leaf balance pressures (figure 19). As leaf balance pressures increased above 2 MPa, shoot balance pressures increased more rapidly with increasing water deficit than did that of leaves from the shoot. By the time leaf balance pressures had risen to 4 MPa shoot balance pressures were in excess of 6 MPa, the highest pressure which could be measured with the gauges available.

a) Artifacts causing high shoot balance pressures

It was possible that the difference between leaf and shoot balance pressures was an artifact caused by

i) non-equilibration of water potentials within the shoot when balance pressures were determined,

or ii) evaporation from the shoot while in the pressure chamber.

i) Non-equilibration in the pressure chamber

Too fast a rate of pressure increase may result in over-estimation of balance pressure which subsequently falls to its correct value as equilibrium is attained (Waring and Cleary, 1967; Boyer and Gorashy, 1971). In table 3 are presented the results of experiments in which Rhododendron shoots were

brought to their apparent balance pressures at a rate of pressure increase of .002 - .003 MPa s^{-1} and subsequently held at pressures just below this for periods of from several minutes to several hours. At intervals the pressure was increased again to obtain the balance pressure.

It was found (table 3) that shoot balance pressure increased at a rate of 0.1-0.4 kPa s^{-1} over the period for which the shoot was held near its balance pressure.

Table 3. Apparent balance pressures of Rhododendron shoots held at just below the pressure at which sap appears at the end of the stem. Time is taken from that of the first appearance of sap at the cut end of the stem.

Shoot number							
1		2		3		4	
Time (min)	P (MPa)	Time (min)	P (MPa)	Time (min)	P (MPa)	Time (min)	P (MPa)
0	3.45	0	3.98	0	3.45	0	3.2
4	3.60	7	4.10	7	3.53	40	3.4
6	3.60	9	4.20	16	3.55	60	3.7
8	3.70					130	4.05
11	3.75					180	4.45
13	3.75						
22	3.80						

The results of similar experiments using Rhododendron leaves have been shown in figure 17.

As shoot balance pressures increased rather than decreased when the shoot was held at pressure, it appears that the difference between leaf and shoot balance pressures was not the result of inequilibration during balance pressure measurements.

ii) Evaporation from the shoot

Evaporation from the shoot in the pressure chamber could result in a fall in Ψ_l and hence in higher shoot balance pressures.

Weight losses by shoots during balance pressure determinations were determined in three cases. These showed that 10-15g shoots lost a mean of 0.3g in weight over this period.. This was about 4% of the turgid water content of these shoots. However, the stem held at near its balance pressure for three hours (table 3) was found to have lost 1.47g in weight over this period, amounting to 17% of the water content of the turgid twig.

In Rhododendron leaves a 4-5% fall in RWC will increase leaf balance pressure by about 0.2 MPa, a 17% decrease by about 0.5 MPa (figure 14). By extending these findings from leaves to shoots it appears that evaporation from shoots was insufficient to account for the observed increase in shoot balance pressure.

Increasing amounts of gas were found to be blown through the stems of shoots as chamber pressure increased and may have caused some of the evaporation occurring during balance pressure measurements.

However, evaporation rates were insufficient, even in shoots maintained at high pressure, to account for the magnitude of the differences between leaf and shoot balance pressures which were found.

Evaporative water losses increase balance pressures by lowering cell Ψ, including that of the leaves. Therefore balance pressures of leaves on the shoot would be expected to increase during balance pressure measurements of the shoots if the higher shoot balance pressures were due to evaporation. The balance pressures of leaves sampled before or after the shoot balance pressure was measured were compared (table 4) to see if this occurred.

Table 4. Balance pressures of leaves taken from shoots before or after the shoot balance pressures were taken.

Experiment	Twig P (MPa)	Leaf P (MPa) (before twig P taken)	Leaf P (MPa) (After twig P taken)
1	2.96	2.04	2.17
2	3.10	1.97	1.97
3	2.00	1.64	1.65
4*	3.25	2.66	3.01
14	1.06	1.38	1.15
15	1.81	1.68	1.66
20	2.24	2.51	2.28
21	3.07	2.29	2.36
22	2.84	2.17	2.41
23	3.60	2.62	2.66
25	2.11	1.88	1.98

* Held at near balance pressure for three hours.

In seven of eleven experiments leaves sampled after shoot balance pressure measurements had higher balance pressures than those sampled before. Moreover, in these seven instances the increases found were insufficient to account for the observed differences between leaf and shoot balance pressures.

Bearing these observations in mind, the difference between leaf and shoot balance pressures was thought to result from factors affecting the partitioning of water within the tissues of the shoot.

b) Voids causing high shoot balance pressure

i) Pre-existing voids in the shoot

As the difference between leaf and shoot balance pressure did not become marked until sap tension had increased to at least 2 MPa (figure 19),

it was unlikely that the difference was due to the filling of large voids existing in a turgid or moderately stressed shoot. Such voids, be they intercellular spaces or empty xylem conduits, constitute relatively large diameter capillaries which will empty of sap as Ψ_l falls over the first few tenths of a megapascal.

The extra pressure required to express the sap needed to fill these spaces before sap returns to the end of the shoot will therefore be necessary even when the shoot has been subjected to little water stress (Scholander et al., 1965). Moreover, as the volume of voids of this type is fixed, no great changes in the extra pressure required is to be expected as the shoots become progressively more water stressed. The early onset of differences between shoot balance pressure and Ψ_l resulting when pre-existing void spaces in the shoot must be filled before sap returns to the end of the shoot has been demonstrated in Rhododendron roseum shoots (Boyer, 1967). Moreover, Boyer was able to see sap expressed from the xylem entering the pith of R. roseum, a flow which was not observed in these experiments with R. ponticum.

The slightly higher shoot than leaf balance pressures found at balance pressures between 0 and 2 MPa (figure 19) may have resulted from the filling of the small voids created as sap retreated from the cut end of the stem as the shoot dried from full turgor.

However, as the difference between R. ponticum shoot and leaf balance pressures increased markedly as sap tension rose above 2 MPa (figure 19), it was apparent that the void volume in these shoots was increasing as water deficits increased. Such increases in void volume will occur if xylem conduits are progressively emptied by cavitation. However, the differences in leaf and shoot balance pressure may also occur if water expressed by gas pressure acting on one group of cells flows to rehydrate other cells which are at a lower pressure, for instance in the stem protruding from the pressure chamber (Waring

and Cleary, 1967).

ii) Increases in void volume caused by cavitation

Cavitation in shoots and leaves of R. ponticum is known to occur at sap tensions of 1.4 - 2.5 MPa (section 3.5). This range of sap tensions includes that at which the difference between leaf and shoot balance pressures is seen (fugure 19).

The volume of the xylem luminae in the R. ponticum shoots used in these experiments was not measured. However, Rivett (1920) estimates that the lumina constitute about 20% of the xylem cross-section in similar R. ponticum shoots.

It has been shown that even the small xylem volumes of seedlings may contain sufficient water to cause marked changes in cell water potentials. Cary et al. (1968) showed that Ψ of corn and tomato seedling increased by up to 0.25 and 0.1 MPa respectively when the xylem was deliberately embolised (by cutting the stem) to make the xylem water available to the cells at atmospheric pressure.

Water contained in the relatively large lumen volume of a Rhododendron shoot may therefore be sufficient to keep Ψ_1 high (nearer to zero) and cause the observed differences between leaf and shoot balance pressures.

iii) Rehydration of cells by water expressed from elsewhere in the shoot

It was found that gas escaped through the xylem of Rhododendron shoots in the pressure chamber. Therefore, steep gradients in gas pressure existed in the stem xylem with pressure being at that of the chamber at one end and at atmospheric at the other end. This gradient in gas presure, and mechanical support of the xylem itself, may be sufficient to maintain the xylem pith at a lower pressure than that to which the cells of the leaves and epidermis are subjected. Consequently, Ψ of the pith cells may be lower than that of cells, e.g. in the leaf, which are exposed to the full chamber pressure (Tyree and Hammel, 1972). As the pith and leaf cells are in hydraulic contact through the xylem and cell wall water, water may therefore flow from

the cells at chamber pressure to the pith which is at lower pressure.

In this case the extra pressure required to return water to the cut end of the stem will depend on the volume and desorption characteristics of both the pith and the cell populations donating water to the pith as well as on the pressures to which each is subjected (Tyree and Hammel, 1972). The deviation of leaf and shoot balance pressures becomes marked at about the same Ψ as that at which leaf turgor was reduced to zero (about 1.9 MPa, table 7). The difference between leaf and shoot balance pressures may therefore be due to loss of turgor by the cells of the leaves at lower chamber pressure than those of the pith which are protected from the full chamber pressure. Such water exchange between the foliage and the pith has previously been invoked as an explanation of the differences found between the balance pressures of needles and twigs of several species of conifers (Ritchie and Hinckley, 1971).

On the basis of the evidence obtained using Rhododendron ponticum it was impossible to determine whether cavitation or water uptake by partly pressurised pith cells was responsible for the observed differences between leaf and shoot balance pressure. However, it was considered most unlikely that the difference was due to voids existing in shoots before they were subjected to water stress.

3.4.3. The effect of cavitation on pressure chamber measurements of Ψ_l

a) Leaves from plants in the field

The results of experiments (described in section 2.4.2) in which balance pressures and thermocouple psychrometer measurements of Ψ_l were compared are shown in figure 20. Ψ_l determined by the pressure chamber and by psychrometry are in reasonable agreement over the range of leaf balance pressures used in these trials (0 to 3.5 MPa).

Most of the variation about the line of equality of pressure chamber and psychrometer measurements of Ψ_l was probably due to variations in psychrometer

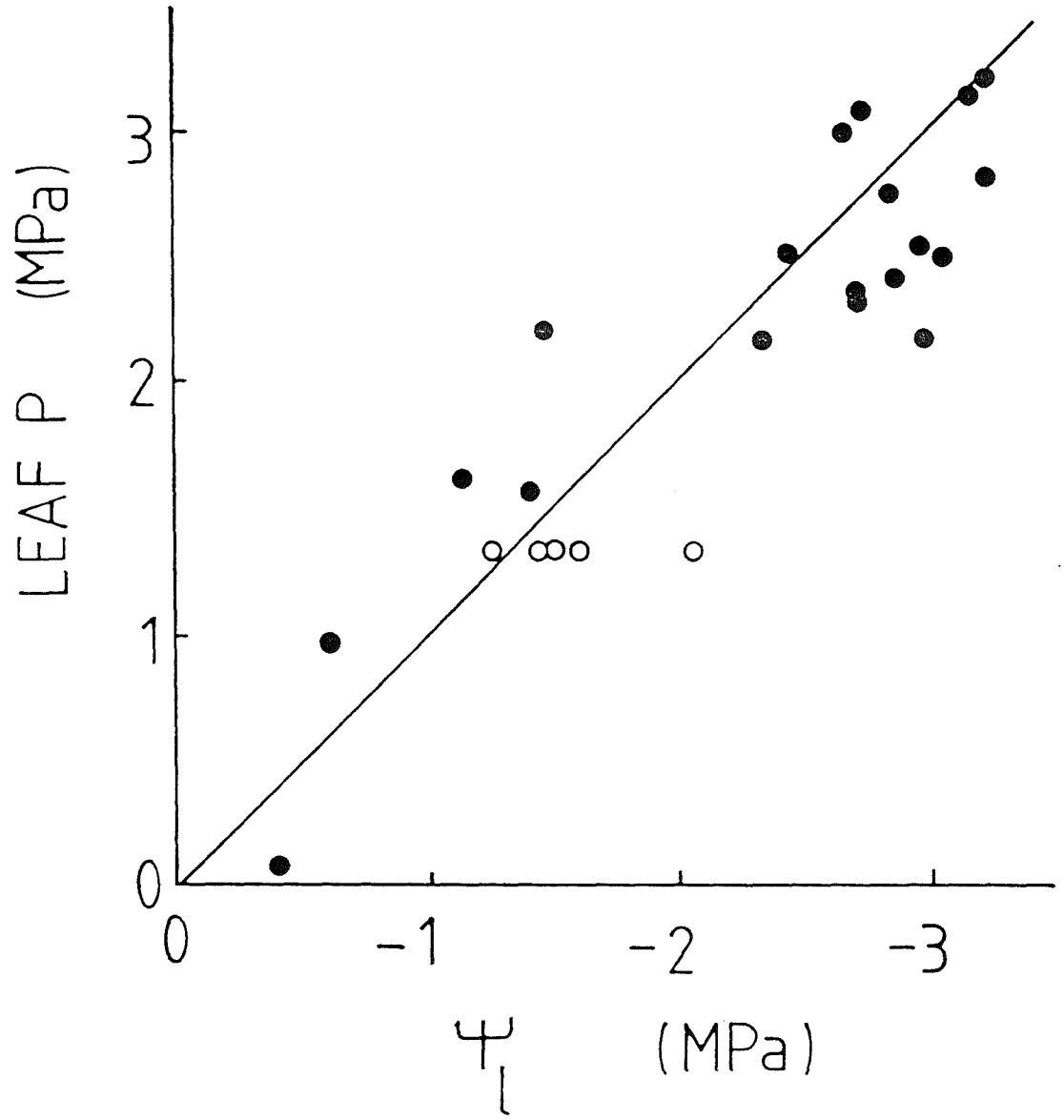


Figure 20. 'Field' Rhododendron. Comparison of leaf balance pressure (P) and leaf water potential (Ψ_l) determined psychrometrically. All leaves were from water stressed shoots. Open symbols indicate five samples from the same leaf. The diagonal line indicates a 1:1 relationship between P and Ψ_l.

measurements. Psychrometer measurements of Ψ_l of several leaf discs cut from the same leaf showed differences of up to 1.5 MPa in Ψ_l between samples (figure 20).

No sudden change in the relation of balance pressure to Ψ_l which might be caused by cavitation was found (West and Gaff, 1971).

The equality of balance pressure and psychrometer measurements of Ψ_l suggests that in Rhododendron leaves

i) balance pressure measurements yielded good estimates of Ψ_l

and ii) cavitation of xylem sap did not cause identifiable errors when the pressure chamber was used to measure sap tension.

b) Leaves from plants in the glasshouse

The relation of balance pressure to Ψ_l was also investigated using Rhododendron plants growing in pots in the glasshouse.

Leaves were stressed in one of two ways for this experiment.

i) Starting in late June 1982, plants were denied water and leaves sampled for balance pressure measurements at intervals of several days as stress increased. The leaves were enclosed in small plastic bags for about twenty minutes before removal from the plant to minimise post-excision changes in Ψ_l (Turner, 1981). After obtaining the balance pressure the leaves were sampled for psychrometer determination of Ψ_l.

ii) Leaves were cut from well-watered plants and allowed to transpire on the glasshouse bench before measurement of balance pressure and Ψ_l.

The results of both i) and ii) are included in figure 21.

At low balance pressures Ψ_l was lower than the corresponding balance pressure would indicate. At high balance pressure the opposite was true and Ψ_l was higher (nearer zero) than the balance pressures indicated.

Results were similar whether leaves were stressed on or off the plants.

The relation of balance pressure to Ψ_l obtained in this experiment was different to that found when the same comparison was made using leaves from a plant growing in the field.

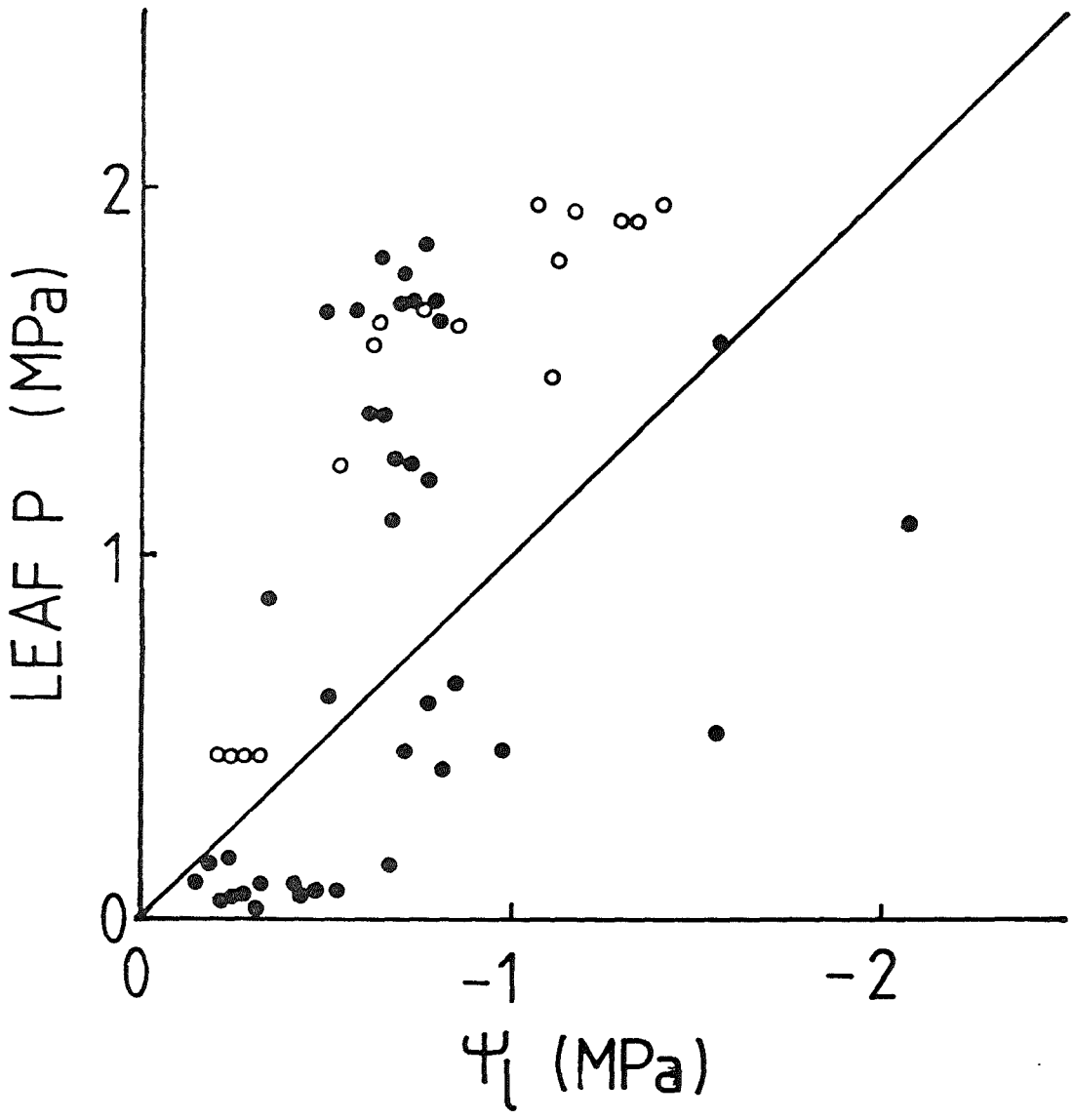


Figure 21. 'Glasshouse' Rhododendron. Comparison of leaf balance pressure (P) and leaf water potential (Ψ_1) determined psychrometrically.

o Leaves from water stressed plants.

• Leaves dried on bench after cutting from well-watered plants.

The diagonal line indicates a 1:1 relationship between P and Ψ_1.

It is possible that the psychrometer values of Ψ_l were in error because of the effects of wounding during sampling. Talbot et al. (1975) have shown that wounding during sampling for the psychrometer may cause Ψ_l to rise (become less negative) by up to 1 MPa. The magnitude of this effect seems to depend on both the species and the ratio of cut surface to sample volume.

The leaves from plants in the glasshouse were less glabrous, smaller and thinner than those of plants in the field. As a result, leaf discs punched from leaves of glasshouse plants often had ragged edges, whereas those from the field plants, being thicker and stiffer, were cut more cleanly. The higher ratio of damaged cell surface area to total sample volume in the leaves from glasshouse plants may have been responsible for the appearance of wounding effects on Ψ_l in leaves from the glasshouse plants, but not in the leaves from the field.

c) Psychrometer determinations of turgor

Calculations of leaf turgor (Ψ_p) were made using psychrometer data obtained using glasshouse plants. The results were very scattered and, because of the effects of wounding during sampling on Ψ_l, may bear little resemblance to those occurring in healthy leaves. For these reasons the Ψ_p values calculated in this manner could not be compared with Ψ_p values obtained from pressure chamber measurements and will not be discussed further.

d) Osmotic potential of xylem sap

i) From turgid leaves

Balance pressures may be approximated to Ψ_l if xylem sap Ψ_s is near zero (Duniway, 1971; Boyer, 1967). Determinations of Ψ_s of sap expressed from leaves of each of the species for which the relation of balance pressure to RWC was derived were carried out. The results are presented in table 5.

Table 5. Osmotic potential of xylem sap expressed from turgid leaves of the species used for acoustic experiments.

Species	Ψ_s (MPa) (± S.E.)	Number of samples	Method used for determination
Acer	−0.14 ± .07	6	FPO
Alnus	−.07 ± .03	4	TCP
Eucalyptus	−.07 ± .00	3	FPO
Fraxinus	−.06 ± .02	3	TCP
Lycopersicum	−.06 ± .01	2	TCP
Plantago	−.22 ± .08	3	TCP
Rhododendron	−.07 ± .14	13	TCP
Ricinus	−.07 ± .02	2	TCP

FPO - Freezing point osmometer.
TCP - Thermocouple psychrometer.

The accuracy with which psychrometric determinations of Ψ_s can be made declines as Ψ_s rises above -0.1 MPa toward zero. However, values of Ψ_l measured in this range were considered accurate enough to indicate that Ψ_s of the sap samples were in the range 0 - -0.1 MPa.

The only species found to have sap Ψ_s of below -0.1 MPa were Acer and Plantago. It was almost certain that sap collections from Plantago were contaminated by exudate from cut cells at the end of the fleshy petiole.

As xylem sap Ψ_s was near zero in most of the species tested, balance pressure measurements were used to estimate Ψ_l in these species (Duniway, 1971; Boyer, 1967). Even in those species in which sap Ψ_s was less than -0.1 MPa (in Acer and Plantago) the errors in Ψ_l due to Ψ_s of the sap would be a relatively small fraction of the total in severely water stressed leaves and so were neglected.

ii) From water stressed leaves and shoots

The sap Ψ_s values presented in part (a) of this section were obtained by expressing sap from turgid leaves. However Boyer (1967) has reported that sap Ψ_s may change as samples become water stressed. Such a change may affect the use of the pressure chamber for making measurements of Ψ_l. Changes in Ψ_s of xylem sap in response to imposed water stress were examined using Rhododendron shoots which had developed water stress by transpiring on the laboratory bench. The results are presented in figure 22.

Ψ_s is between -.01 and -.03 MPa until leaf water potential falls below approximately -2 MPa. At Ψ_l lower than -2 MPa sap Ψ_s declines rapidly, reaching -0.18 MPa as Ψ_l falls to about -2.5 MPa. Even at Ψ_l of -2.5 MPa Ψ_s is still a small part of Ψ_l. However, if Ψ_s continues to fall with Ψ_l a diversion of Ψ_l determined by the psychrometer and by the pressure chamber will eventually become evident. However, such a difference had not yet occurred when Ψ_l had fallen to -3.5 MPa (figure 20).

It was concluded that for Rhododendron pressure chamber measurements of Ψ_l were unlikely to be in error even at low Ψ_l (high balance pressures) because of changes in the osmotic potential of the xylem sap.

3.4.4. Pressure-volume (P-V) curve analysis of the relation of balance pressure to RWC

Noises similar to the clicks attributed to cavitation have been reported to have occurred in wilting leaves even when the sap is not in tension (Milburn, 1973a).

Before conducting experiments to investigate the relationship between clicks and cell turgor it was necessary to find the water potentials at which the leaves used in acoustic experiments lost turgor. This could not be found by psychrometry, at least in Rhododendron (section 3.4.3), so P-V curve analyses of balance pressure data already obtained for these species (section 3.3) were undertaken.

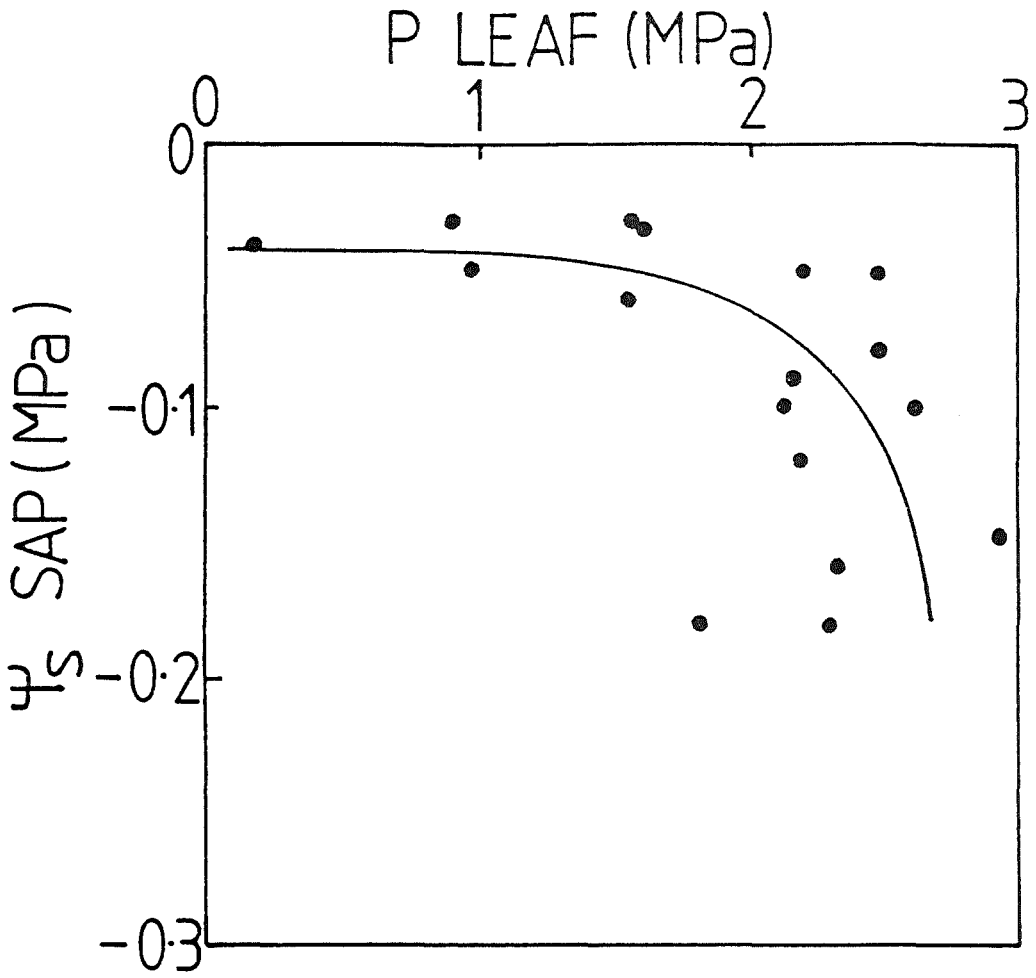


Figure 22. Osmotic potential (Ψ_s) of xylem sap expressed from Rhododendron leaves and shoots of balance pressures between 0 and 3 MPa.

The P-V curve analyses were carried out assuming that leaf balance pressures were an accurate reflection of Ψ_l (section 3.4.3).

These analyses were made using plots of the inverse of balance pressure ($^1/_p$) against RWC (figure 23). The results are presented for all species in table 6 and for Rhododendron at different times of the year in table 7.

The first value obtained (by inspection of the pressure-volume P-V curve plots) was Ψ_p', the water potential at which cell turgor was first reduced to zero, sometimes called the point of incipient plasmolysis (Tyree et al., 1975) The second and third values, $\Psi_s°$, the solute potential of leaf cells at full turgor, and RWC', the fraction of apoplastic water, were then found by fitting a linear least squares regression line to the points at RWC lower than that at Ψ_p'. $\Psi_s°$ was found from the intercept of the line with the y-axis (at 100% RWC) and RWC' by the intercept on the x-axis (at $^1/_p = 0$).

Tables 6 and 7 also present the ratio of leaf dry weight to turgid weight for each species.

An obvious feature in these analyses was the frequency with which negative apoplastic water contents were calculated and the sometimes very low regression coefficients for the lines relating $^1/_p$ to RWC. Only three species, Alnus, Lycopersicum and Rhododendron, were found to give positive RWC'. The low regression coefficients were in part due to low numbers of balance pressure determinations at low Ψ_l, and much of the difference must be attributed to variability between leaves in the samples.

In Rhododendron there was a decrease in Ψ_p' with the onset of winter in both years during which samples were collected. This amounted to a 0.5 MPa fall (from -1.79 to -2.34 MPa) between December 1979 and January 1980 in the Ψ_l at which cell turgor became zero, and 0.2 MPa (-1.79 to -2.03 MPa) between June 1980 and January 1981. Over the same periods $\Psi_s°$ fell by 0.26 and 0.05 MPa respectively. The apoplastic water fraction (RWC') increased with leaf age in both years, as did the dry weight : turgid weight ratio (remembering that the change to the new season's leaves in samples was between July and October).

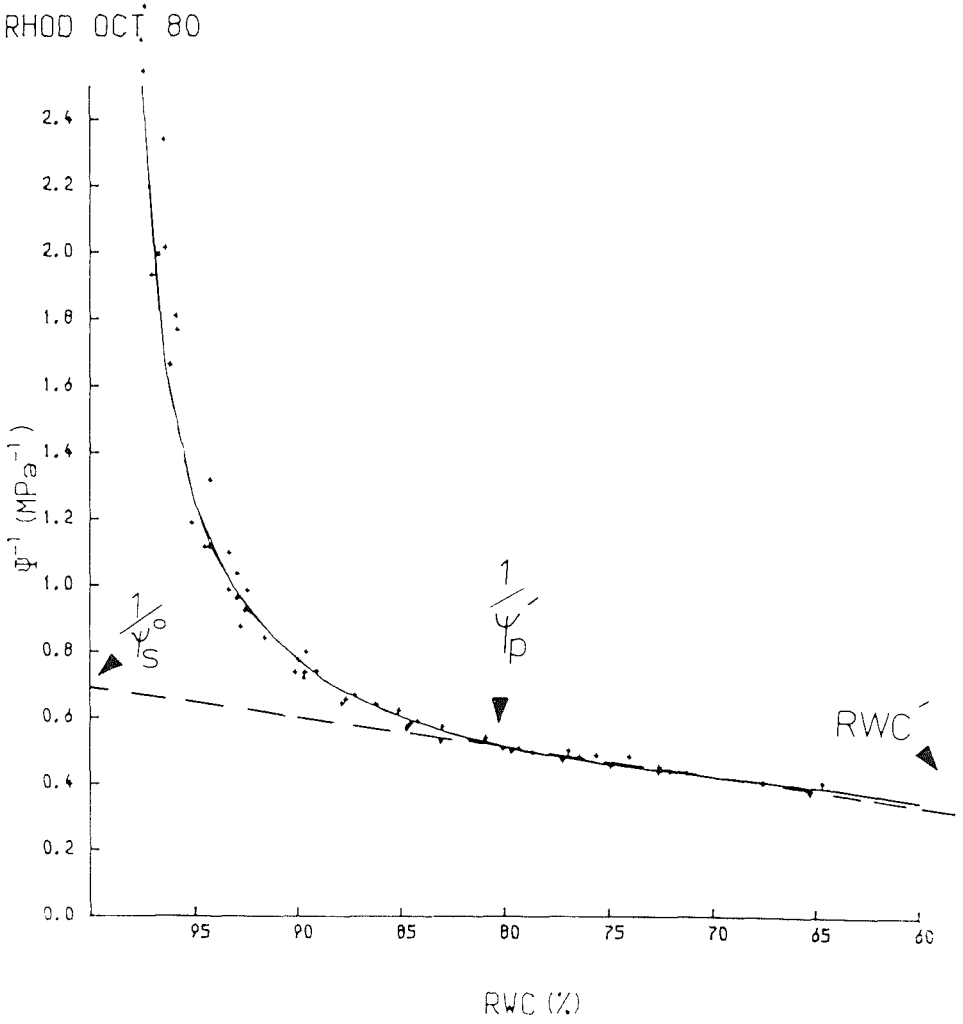


Figure 23. Calculation of water relations parameters from inverse pressure-volume (P-V) curves. The point of incipient plasmolysis (Ψ_p') was found first by identifying the junction of the linear and curved parts of the P-V curves. Initial osmotic potential ($\Psi_s°$) and apoplastic water content (RWC') were found from a least-squares regression fitted to points of Ψ less than Ψ_p'. The data shown were obtained from Rhododendron leaves collected in October, 1981.

Immature Rhododendron leaves did not form a very uniform group. This was because, while the immature leaves were softer, smaller and lighter in colour than the older 'mature' leaves, there was considerable variability in development between individual leaves within this general group. Within the group of 'immature' leaves the relatively high Ψ_p' (-1.00 MPa) and low dry weight : turgid weight ratio (20%) are notable.

The results of these analyses, and especially the values of $\Psi_s°$ and RWC', may be in error for several reasons. Ψ_p' was chosen from an assessment of the relation of $^1/_p$ to RWC and is therefore subjective (Tyree and Hammel, 1972).

The values of $\Psi_s°$ and RWC' depend on the value chosen for Ψ_p' as the line from which they are calculated was fitted through points at Ψ_1 below Ψ_p' (figure 23).

The sometimes very low regression coefficients of the line relating $^1/_p$ to RWC was due in part to variability between the desorption isotherms of individual leaves and also to fitting a regression line through a relatively low number of points.

To remedy the above problems it is necessary to construct the pressure-volume curves using single leaves or shoots in a Hammel-type expression experiment (e.g. Tyree et al., 1973 ; Tyree et al., 1978), by in situ monitoring of changes in Ψ_1 of a transpiring shoot (Richter, 1978) or by frequent sampling for Ψ_1 measurement from a shoot (Talbot et al., 1975).

However, it was possible that the low, sometimes negative values of RWC' which were found may have resulted from over-estimation of Ψ_1 by the pressure chamber at high leaf water stress. The fall in Ψ_s of xylem sap expressed from Rhododendron leaves and shoots as Ψ_1 falls below -2 MPa is an indication that this might occur.

It is known that cell membranes may be damaged by water stress (Simon, 1974) and that as a result they become increasingly permeable to electrolytes (Scherbakova and Kacperska-Palacz, 1980; Leopold et al., 1981). Alternatively, cell membranes may be damaged by conditions in the pressure chamber (e.g. Tyree et al., 1978) with similar results.

However, Scholander et al. (1964) were able to construct apparently correct (i.e. without finding negative RWC') P-V curves for a number of species despite increasing sap solute concentration. This aspect of pressure chamber technique requires further investigation.

After considering these problems in carrying out P-V curve analyses Ψ_p' values were probably accurate enough to be used in investigations of the relation of the loss of turgor to click production.

However, values of RWC' and Ψ_s were often very unreliable.

Table 6. Pressure-volume curve parameters obtained using balance pressure data of many leaves (section 3.3).

$\Psi_s°$ = Symplastic solute potential at full turgor.

Ψ_p' = Point of incipient plasmolysis.

RWC' = Apoplastic water content (RWC).

F = Leaves from plants in the field.

G = Leaves from plants in the glasshouse.

Leaf class	Ψ_p' (MPa)	$\Psi_s°$ (MPa)	RWC' (%)	r^2 of linear regression (%)	Leaf Dry Weight / Leaf Turgid weight (% ± S.E.)
Acer (F)	-1.23	-1.29	-21.2	50	33.3 ± .5 (n = 36)
Acer (G)	-1.07	-1.29	-29.7	40	22.2 ± 0.3 (n = 23)
Alnus (F)	-1.95	-1.60	10.7	60	38.7 ± 0.2 (n = 29)
Eucalyptus (G)	-1.40	-1.26	-13.3	76	31.4 ± 0.3 (n = 26)
Fraxinus (F)	-2.10	-2.18	-14.8	57	33.4 ± 0.5 (n = 38)
Lycopersicum(G)	-0.69	-0.61	17.2	64	6.3 ± 0.1 (n = 39)
Plantago (F)	-1.03	-1.18	-111	13.5	15.1 ± 0.2 (n = 12)
Plantago (G)	-1.18	-1.31	-.91	45	15.1 ± 0.8 (n = 21)
Rhododendron(F) (October 1980)	-2.03	-1.08	-15.5	90	34.6 ± 0.3 (n = 25)
Ricinus (G)	-0.57	ND	ND	ND	13.6 ± 0.7 (n = 12)

Table 7. Pressure-volume curve parameters obtained using balance pressure data of Rhododendron leaves collected at intervals over a two-year period (the month and year of each collection are indicated). Abbreviations are as for table 7.

All collections were from the same large bush in the grounds of the Garscube Estate, Glasgow.

Collection date	Ψ_p' (MPa)	$\Psi_s°$ (MPa)	RWC' (%)	r^2 linear regression (%)	Leaf Dry Weight / Leaf Turgid Weight (% ± S.E.)
December 1979	-1.79	-1.38	44.1	65	34.8 ± 6.9 (n = 10)
January 1980	-2.34	-1.62	61.3	42	37.5 ± 0.5 (n = 16)
April 1980	-2.23	-1.66	56.9	83	37.6 ± 0.5 (n = 18)
June 1980	-1.79	-1.56	8.1	33	41.4 ± 0.6 (n = 15)
October 1980	-2.03	-1.08	15.5	90	34.6 ± 0.3 (n = 25)
January 1981	-2.03	-1.61	37.8	87	37.4 ± 0.5 (n = 28)
April 1981	-1.93	-1.57	36.3	86	39.7 ± 0.7 (n = 20)
July 1981	-1.84	-1.60	4.5	76	39.9 ± 0.8 (n = 29)
Immature leaves (July 1981)	-1.00	-0.94	-32.7	17	20.2 ± 0.5 (n = 8)

3.5. Click frequency and sap tension

3.5.1. Introduction

The use of the acoustic technique to detect cavitation and of the pressure chamber to relate sap tension to changes in leaf water content have already been described (sections 3.2, 3.3 and 3.4).

The sap tensions in cavitating leaves can then be found by comparing the RWC of these leaves against the 'calibration' of sap tension against RWC for similar leaves.

Cavitation can also be related to sap tension more directly by using the pressure chamber to measure sap tensions in the cavitating leaves themselves. Two methods were used:

a) leaves were removed from the acoustic detector while clicks were being detected and their sap tension determined using the pressure chamber.
b) the pressure chamber was used to control sap tensions in a leaf while cavitation was monitored using the acoustic detector.

3.5.2. Direct measurement of sap tension in cavitating leaves

In figures 24 and 25 are shown the results of experiments in which sap tensions in cavitating Rhododendron leaves or shoots were measured using the pressure chamber.

The pressure chamber was used to measure the sap tensions in a) leaves mounted directly on the acoustic detector and b) in leaves taken from a shoot mounted on the detector.

a) Cavitating sap tensions in Rhododendron leaves

Rhododendron leaves were mounted on the acoustic detector and removed and their balance pressures measured when i) clicks had just begun, ii) click frequency was near maximal and iii) when click frequency had declined from the maximum.

The patterns of click frequency and the sap tensions in the leaves when

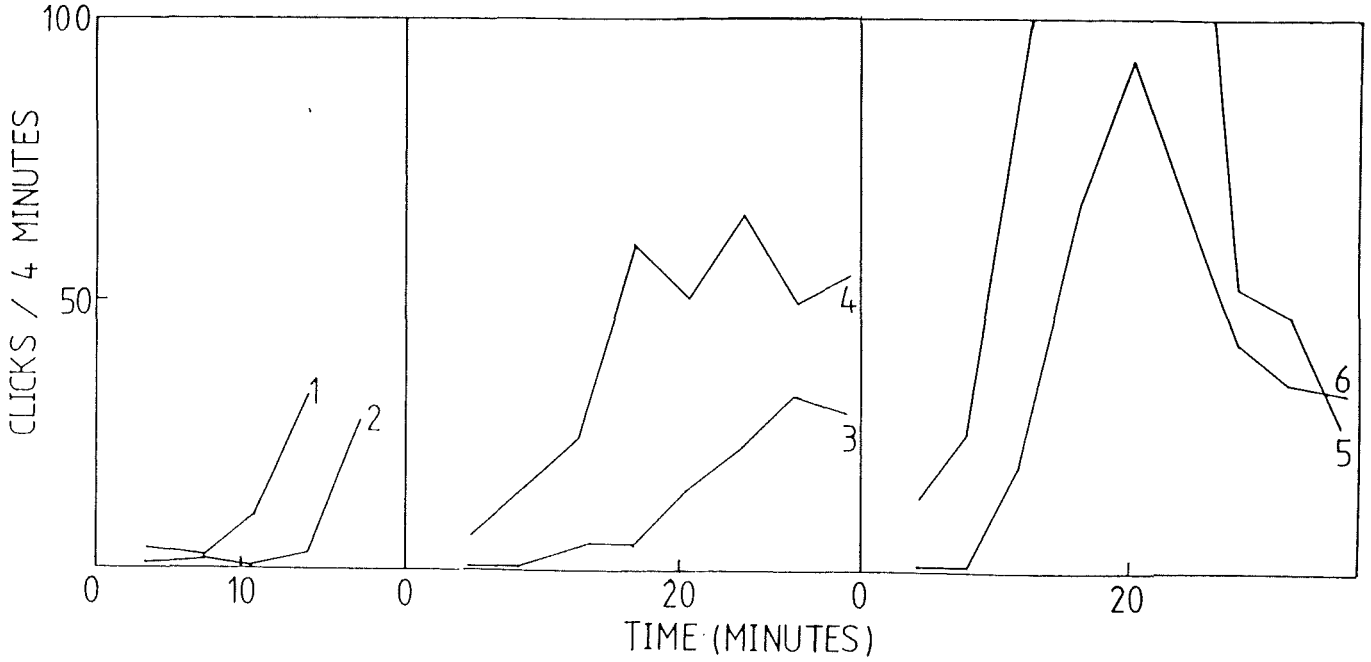


Figure 24. Determination of sap tensions causing cavitation by finding the balance pressures of cavitating leaves. Click production in Rhododendron leaves was monitored and leaf balance pressures determined when click frequency was a) rising, b) near maximal or c) falling. The balance pressures obtained were

Click frequency:	a) rising	b) near maximal	c) falling
	Leaf 1 = 1.41 MPa	Leaf 3 = 1.76 MPa	Leaf 5 = 2.72 MPa
	Leaf 2 = 1.74 MPa	Leaf 4 = 2.10 MPa	Leaf 6 = 2.52 MPa

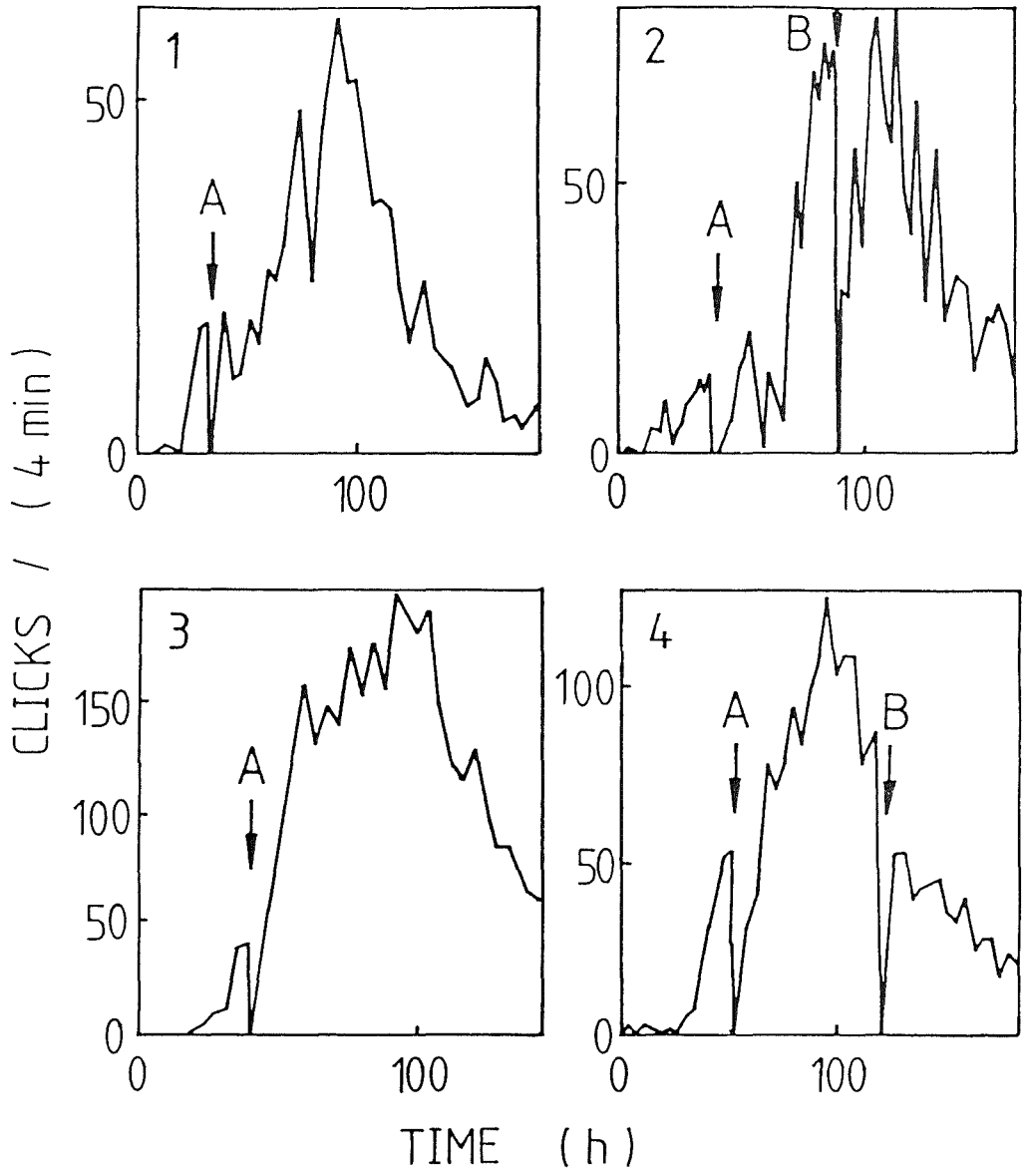


Figure 25. Determination of sap tensions causing cavitation by removing leaves from cavitating shoots. Rhododendron shoots were collected in February 1981 and mounted on the acoustic detector. Click production was monitored as the shoots dried. At the times indicated by arrows leaves were removed for balance pressure determinations.

The balance pressures found were:

Shoot 1		Shoot 2		Shoot 3		Shoot 4	
A	1.2 MPa	A	1.41 MPa	A	1.55 MPa	A	1.43 MPa
End	2.2 MPa	B	1.86 MPa	End	2.1 MPa	B	1.92 MPa
		End	2.1 MPa			End	2.1 MPa

the experiments were terminated are given in figure 24 and its legend. Examination of the petioles of Rhododendron leaves removed from the acoustic detector showed that the vascular strand split longitudinally for a short distance above and below the point of insertion of the needle. Except for conduits adjoining the split other xylem conduits in the vascular strand appeared undamaged. Leaves were not replaced on the acoustic detector after a balance pressure measurement.

b) Cavitating sap tensions in Rhododendron shoots

The pattern of clicks detected in 150-200 mm long Rhododendron shoots transpiring on the acoustic detector are shown in figure 25. At the times indicated leaves were removed and their balance pressures determined. These are presented in the legend to figure 25.

Discussion

Cavitation began and was most frequent at sap tensions of about 1.4 and 2 MPa respectively in both leaves which had been mounted on the acoustic detector themselves or had been cut from shoots on the acoustic detector.

The similar results obtained when leaves were taken from the needle of the acoustic detector or from a shoot (and were therefore free of xylem damage inflicted by the needle of the acoustic detector) are an indication that in Rhododendron balance pressures are little affected by damage inflicted by the needle of the acoustic detector. However the balance pressures of leaves of other species may be affected by mounting on the acoustic detector. Balance pressures of Lycopersicum may be reduced by 0.3 MPa or more by damage inflicted by the needle of the acoustic detector (Nonhebel, pers. comm.). Moreover, removal of the damaged section of the petiole or stem is not possible as errors may also result from this (Ritchie and Hinckley, 1975).

The balance pressures of leaves removed from the acoustic detector can therefore be used to determine the sap tensions at which cavitation occurs if the effect of damage caused by mounting on the acoustic detector is taken into

account.

However, the technique has only limited usefulness in defining the range of sap tensions over which clicks occur because of the subjectivity involved in determining when click frequency is maximal and when the ability of the sample to produce clicks has been exhausted.

3.5.3. Indirect measurement of sap tension in cavitating leaves

Sap tensions characteristic of the relation of cavitation to sap tension in leaves are given for all species in table 8 and for Rhododendron at different times of the year in table 9.

After examining the pattern of click frequency against time (section 3.2.2) four methods by which the relation of cavitation to sap tension might be characterised for each plant species were adopted. These methods are illustrated in figure 26.

a) Construction of a 'cavitation profile' by summing the number of clicks occurring over successive known increments in sap tension.

b) Calculation of the sap tension by which a known fraction of the number of clicks detected during an acoustic experiment were accumulated (10 and 50% fractions were used).

c) Calculation of the sap tension at the time at which click frequency was maximal.

d) 'Averaging' of profiles within a group of experiments to obtain a 'mean' profile for that class of leaves.

The selection of the time at which click frequency was maximal and by which given fractions of the total number of clicks had occurred was straight-forward, as was the use of the calibration of sap tension against RWC to find the sap tension in leaves at these times.

Cavitation profiles were constructed as follows (figure 26).

The calibration curves of sap tension against RWC were used to convert the desired increments of sap tension (usually 0.2 MPa) to increments in RWC.

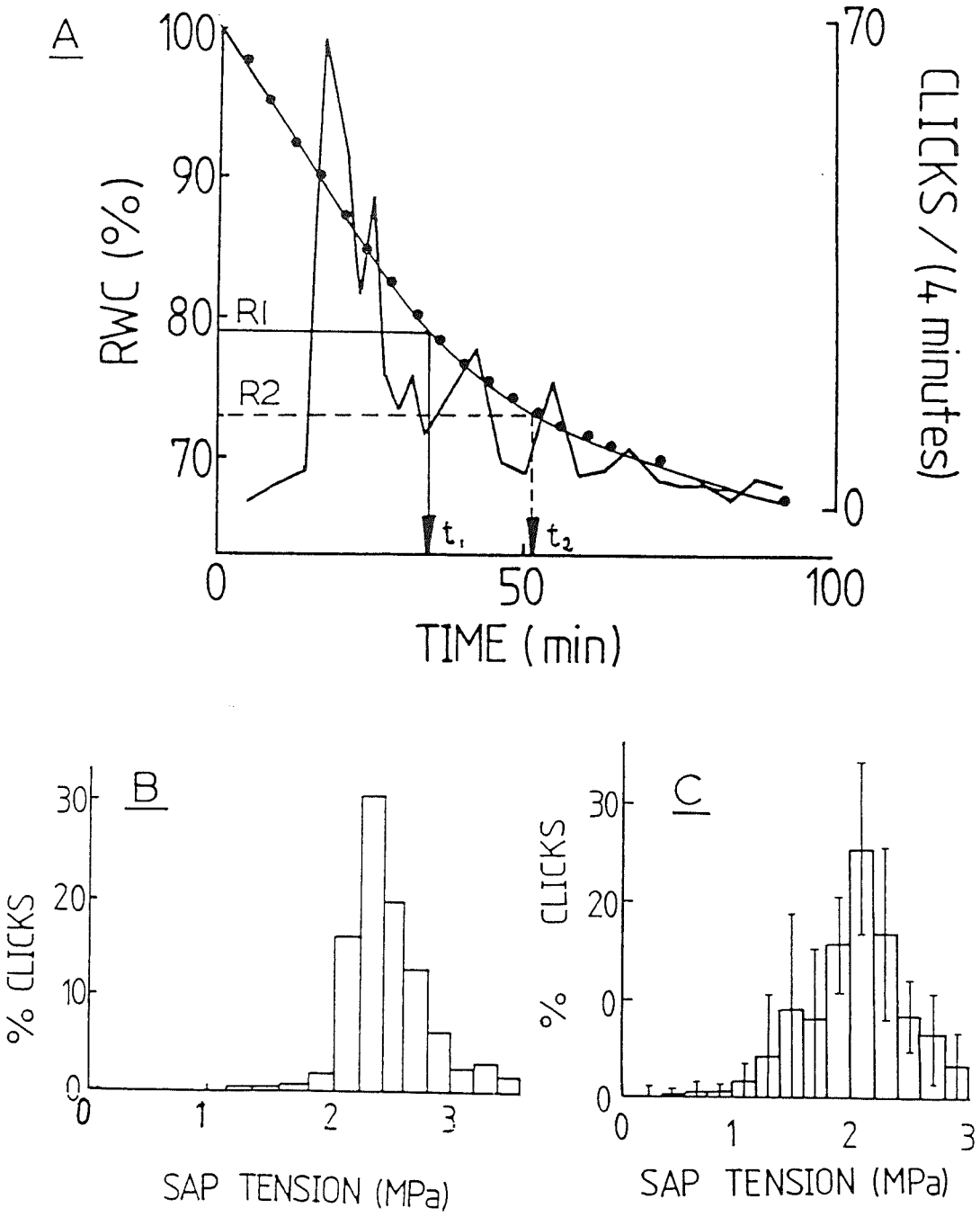
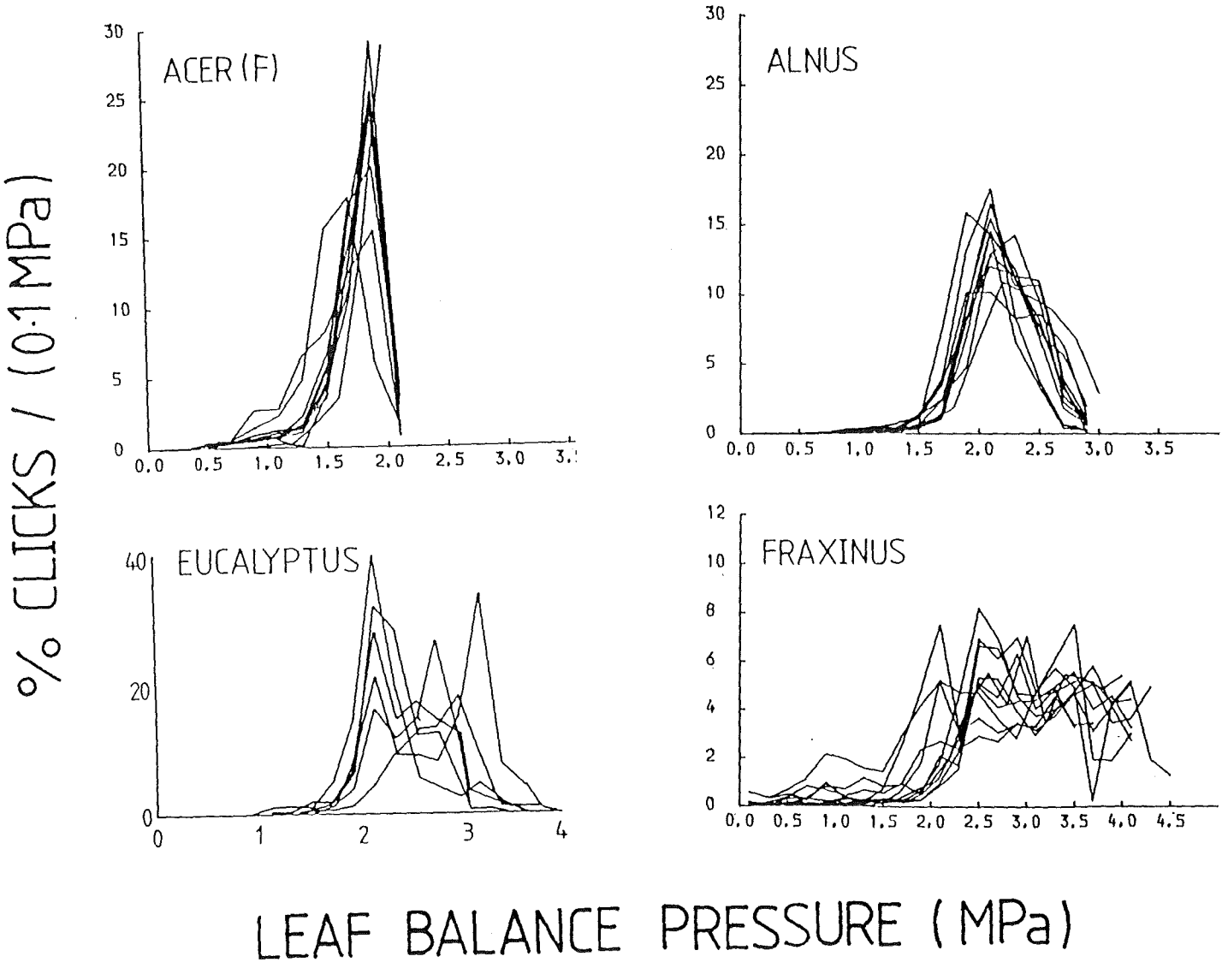
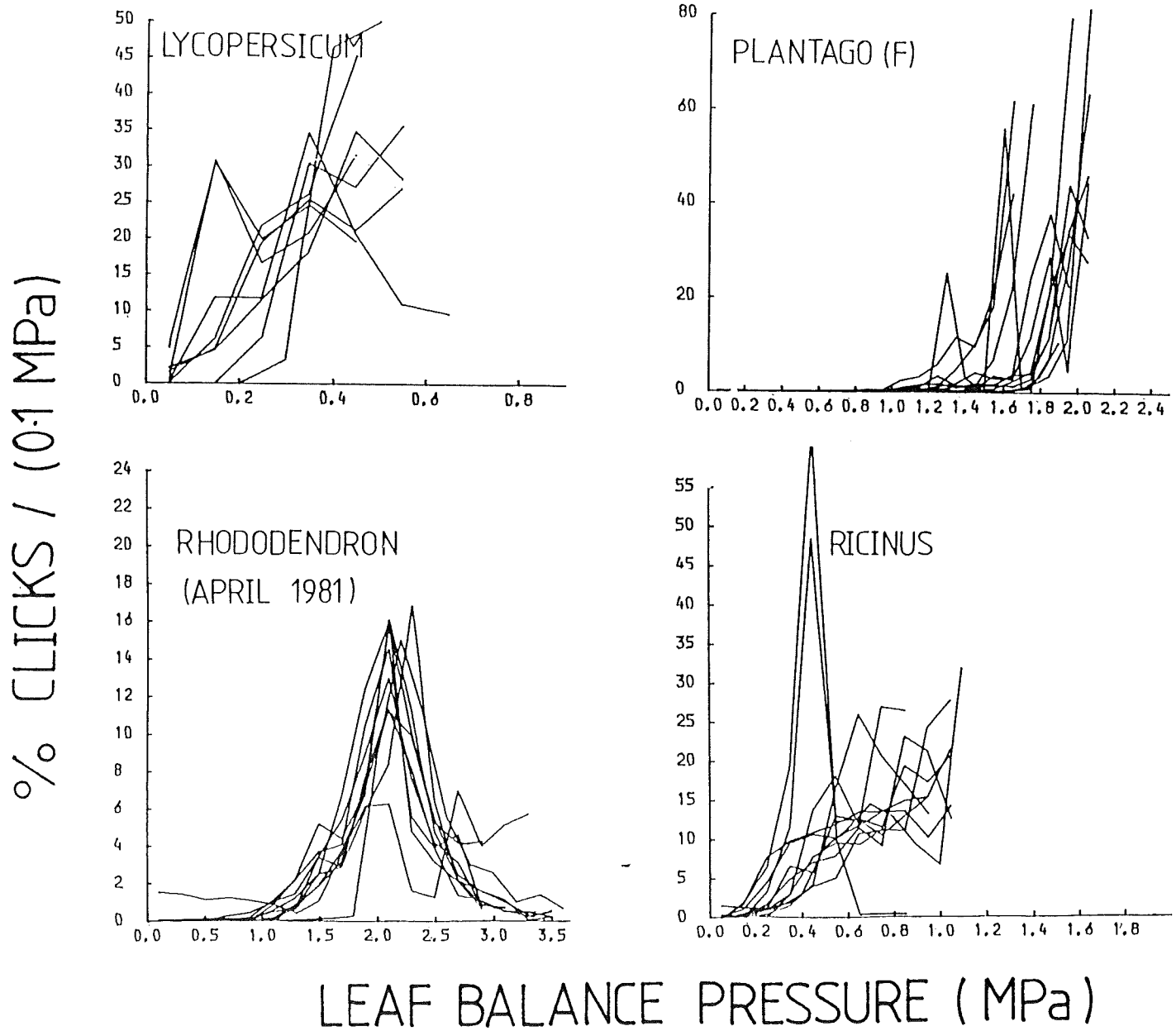


Figure 26. Determination of the sap tensions at which cavitation occurs. The RWC, and hence sap tension, at which clicks begin or are most frequent is found by inspection of plots such as are shown in A. Cavitation profiles (B) are derived by selecting increments of RWC (R_1 - R_2) corresponding to a desired sap tension increment and summing the clicks detected during the time (t_1 - t_2) taken for RWC to change by the desired increment. Several cavitation profiles may be combined to obtain an 'average' profile (C).

Figure 27. (Two pages). Cavitation profiles of leaves of species used in acoustic experiments. Up to twelve profiles are given for each species to show the differences found between profiles of individual leaves. Only one set of cavitation profiles is shown for those species for which two or more collections were made as results were generally similar for all collections. Note that although 0.2 MPa sap tension increments were used when calculating cavitation profiles of the woody species, the proportion of clicks occurring over each increment has been divided by two to enable easy comparisons to be made with the profiles of the herbs which were calculated using 0.1 MPa sap tension increments.


ACER (F)
ALNUS
EUCALYPTUS
FRAXINUS
% CLICKS / (0·1 MPa)
LEAF BALANCE PRESSURE (MPa)


LYCOPERSICUM
PLANTAGO (F)
RHODODENDRON
(APRIL 1981)
RICINUS
% CLICKS / (0.1 MPa)
LEAF BALANCE PRESSURE (MPa)

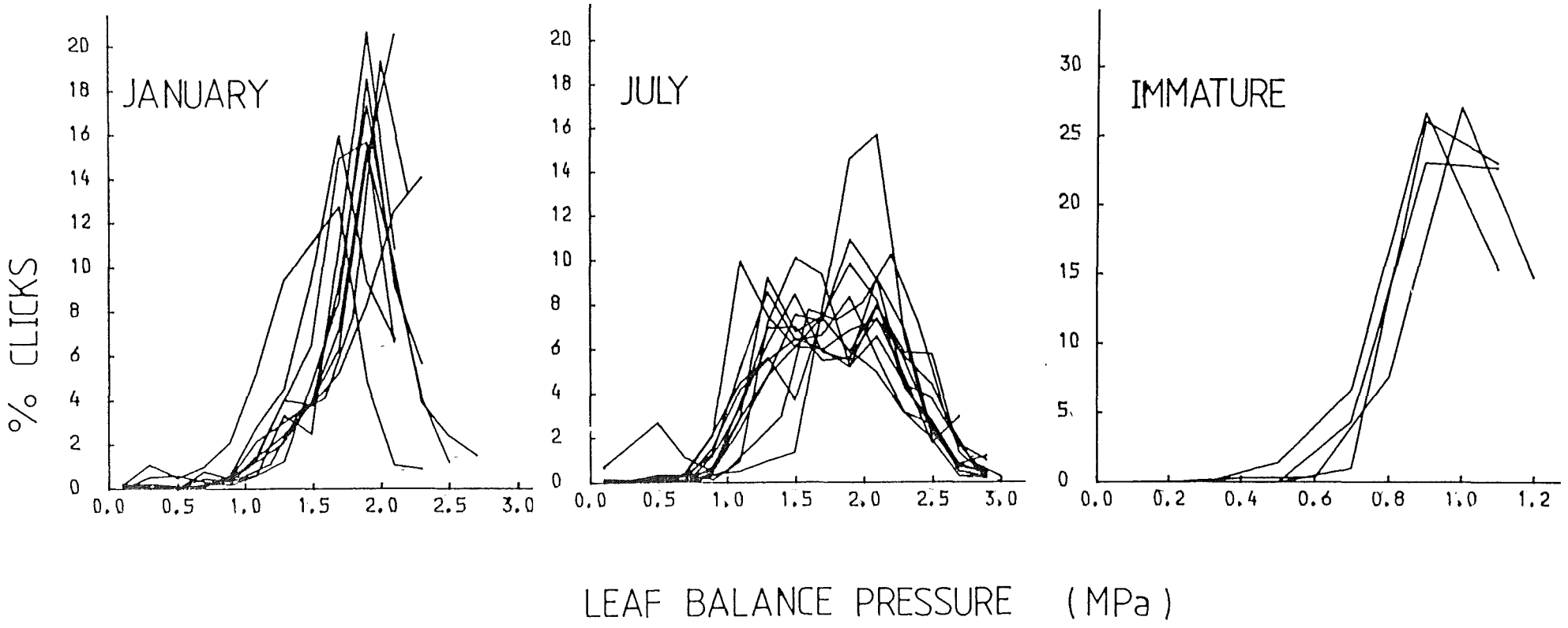


Figure 28. Cavitation profiles of Rhododendron leaves collected in winter (January) or summer (July) and of immature leaves. Note the occurrence of cavitation at lower sap tensions in immature leaves than in either summer or winter collections of mature leaves.

These increments in RWC were then applied to the data of changes in RWC of the leaf on the acoustic detector to find the times between which sap tensions rose by the desired amounts. Clicks recorded over these time intervals were summed and reduced to proportions of the total number for the experiment. The results could be presented as a histogram (e.g. figure 26) although for most purposes line graphs drawn to the midpoints of each sap tension increment sufficed (figures 27 and 28).

Cavitation profiles were 'averaged' by finding the mean proportion of clicks occurring over each tension increment for all experiments in the group. Results were presented as histograms (e.g. figure 26). In order that profiles could be averaged, all profiles had to be calculated over the same range of sap tensions, requiring that a 'cut-off' point to sap tensions be imposed. This limited the maximum sap tensions to which profiles could be calculated.

Discussion

Of the four methods used, the cavitation profile was considered to provide the best description of the relationship between cavitation and sap tension. Inspection of the profiles yielded the sap tensions at which a) cavitation began, b) cavitation was maximal and c) cavitation had ceased. Other methods yielded only point values of sap tensions.

a) Cavitation profiles. Certain features appeared to be common to the cavitation profiles of all species used in these experiments. Cavitation began and reached its maximum incidence at about the same sap tensions in all leaves of the same species or class. This conformity often extended to the proportion of the total number of clicks recorded during the experiment which occurred over each increment in sap tension (e.g. figures 27 and 28).

The shape of the cavitation profiles appeared to be largely unaltered by changes in the size of the sap tension increment used in their calculation (figure 29). Upper and lower limits to the size of the tension increments which could be used were set by the range of sap tensions over which clicks

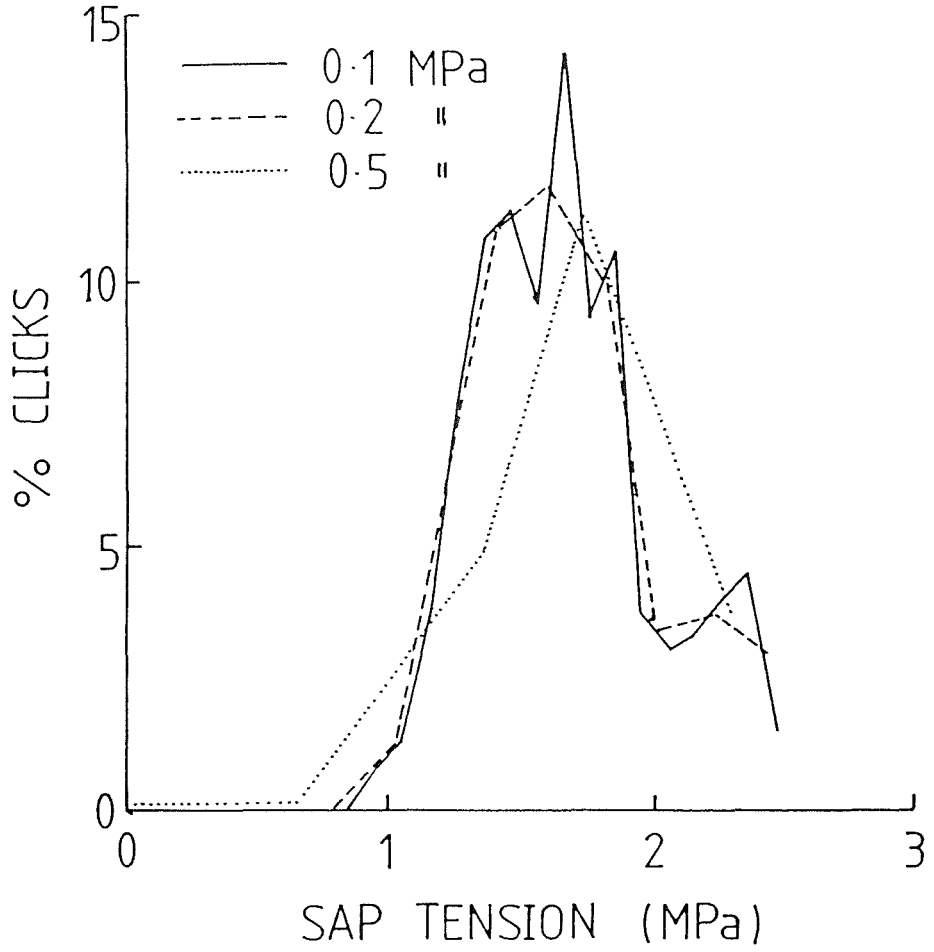


Figure 29. The effect of changing the increments of sap tension used in calculating cavitation profiles on the shape of the profiles obtained.

occurred and by the variability of click frequency about the general trend (section 3.2.2) respectively.

Because of slight differences in the relation of sap tension to RWC, and also differences in the sap tensions at which cavitation occurred between leaves, cavitation profiles of leaves were shifted to slightly higher or lower sap tensions. Consequently averaging of profiles (method d) often resulted in large standard errors for the proportion of clicks which occurred over each sap tension increment (figure 26). It could also result in a misleadingly broad profile if the individual profiles were particularly narrow (e.g. Acer, certain Rhododendron collections) or variable in the sap tensions at which they peaked (Eucalyptus).

Averaging of profiles was therefore of relatively little use when comparing the sap tensions at which cavitation occurred in different species.

A brief discussion of the results obtained for each species is given below.

i) Acer, Alnus, Eucalyptus and Rhododendron all had relatively narrow, symmetrical cavitation profiles with a single, pronounced peak over which 40-80% of the total number of clicks for the experiment were detected. In three species (Acer, Alnus and Rhododendron) cavitation began at sap tensions between 1 and 1.5 MPa and peaked 0.3 to 0.5 MPa higher at around 2MPa. Eucalyptus, whose profiles were more variable than those of the other three species, started to cavitate at sap tensions of 1.5-2 MPa and peaked at around 2.6 MPa (table 8).

ii) Fraxinus was the only species which was found to have rather flat 'plateau type' cavitation profiles which lacked an obvious peak. Cavitation started at sap tensions of 1.5 - 2.0 MPa and continued at a near constant level as sap tensions rose from 2.5 to 4.5 MPa.

iii) Lycopersicum, Plantago and Ricinus were more variable in the shape of their profiles than were leaves of woody species. This was because the estimation of sap tensions was proportionately less precise in leaves of herbs than in trees,

i.e., although cavitation occurred at sap tensions between 0.3 and 1 MPa in herbs (cf. 1.5 - 2.5 MPa in woody plants) sap tension could only be determined to ± 0.2 MPa in both cases. In addition, noise levels often increased in the later stages of experiments using herbs. These noises occurred as leaves wilted and parts of the lamina rubbed together. This was a problem with Lycopersicum and Ricinus. Other tissue noises occurred when parts of the lamina dried to brittleness, a particular problem with Ricinus.

Despite these constraints the tabulated values for sap tensions at the peak of cavitation profiles of these species will not be in error by more than ± 0.2 MPa.

The peaks for Lycopersicum and Ricinus occur at 0.2 and 0.8 MPa respectively. These are about one third of those found in leaves from trees and from Rhododendron. Cavitation profiles of Plantago peaked at sap tensions of about 1.7 MPa, a result more typical of the woody species than of the soft herbs.

iv) Rhododendron leaves sampled at different times of the year.

Mature Rhododendron leaves from all collections began to cavitate at sap tensions of 1.1-1.5 MPa. Their cavitation profiles all peaked at around 2 MPa.

Variability between leaves was greater during the summer than the winter (figure 28). This may have been due to differences in the relation of sap tension to RWC in leaves, perhaps depending on the maturity of each leaf.

Because of limitations in the accuracy with which sap tensions could be determined, experiments using Rhododendron cannot be taken to indicate differences in the sap tensions at which cavitation occurs at different times of the year, despite being subjected to temperatures below freezing in winter (table 9).

Cavitation in immature Rhododendron leaves occurred at sap tensions approximately half those at which cavitation occurred in mature leaves (approximately 0.7 MPa compared to 2 MPa respectively (table 9)).

v) Leaves from plants in the field or in the glasshouse. Characteristic sap tensions and cavitation profiles obtained using Acer and Plantago leaves from plants growing in the field or glasshouse were similar (table 8).

Differences between the characteristic tensions listed for each population in table 8 (up to 0.15 MPa in Acer and 0.3 MPa in Plantago) were believed to be due to inaccuracies arising from use of a single calibration line to relate sap tension to RWC for many slightly different leaves and to the calculation of cavitation profiles over relatively large increments of sap tension. They do not represent real differences in the sap tensions causing cavitation in each group.

The differences in cavitating sap tensions found between species in these studies are therefore believed to be due to the characteristic of each species and not to whether the plants from which they were taken were growing in the glasshouse or in the field.

b) Sap tensions by which 10 and 50% of clicks for the experiment have been recorded

The sap tension by which a small fraction, in this case 10%, of clicks have occurred is an indicator of the minimum sap tensions at which cavitation occurs in a leaf.

The accuracy of the estimate will depend on whether or not the ability of the leaf to produce clicks was exhausted by the end of the experiment. This is best known from a consideration of the cavitation profile of that experiment. As in the majority of species most clicks occur over a narrow range of sap tensions errors in estimating the sap tension at which cavitation starts will probably be small if experiments are continued until the sap tensions at the peak of the cavitation profile have been exceeded.

Errors may be larger if cavitation occurs over a wide range of sap tensions with only a modest fraction of the total click number occurring over each increment of sap tension, as in Fraxinus, or if the decline in click frequency is masked by high levels of noise, as in Lycopersicum and Ricinus.

In such cases it may be better to obtain the lowest sap tensions causing cavitation by inspection of the patterns of click tensions against time rather

than by calculation of the tensions when a small fraction of the total number of clicks have been detected.

The calculation of the sap tensions by which 50% of the potential of the sample to cavitate has been exhausted is subject to similar artifacts as have been described for determinations of minimum cavitating sap tensions by calculation of the tensions by which 10% of clicks have occurred.

The sap tension by which 50% of clicks had occurred was in good agreement with those at which the peak of the cavitation profiles occurred (tables 8 and 9).

c) Sap tension when click frequency is maximal

Sap tensions when click frequency is maximal during an acoustic experiment are in reasonable agreement with those at which cavitation profiles peak in all species except Fraxinus (table 8).

In Fraxinus, which has a plateau-type of cavitation profile (figure 27), sap tensions at the maximum click frequency are at the lower end of the range of sap tensions over which cavitation occurs. This may be the result of a decrease in the rate at which sap tension is increasing, either as the result of a reduction in transpiration rate (section 3.2) or as the rate at which Ψ_1 changes with RWC falls after the loss of turgor in the leaf (figure 14).

The sap tension at which click frequency is maximal was considered to be an acceptable indicator of the sap tension around which the occurrence of cavitation is greatest. The method cannot be used to resolve the range of sap tensions over which cavitation occurs, the determination of which requires the construction of cavitation profiles.

From a consideration of the experiments reported above it appears that the most complete description of the relation of cavitation to sap tension is obtained by construction of a cavitation profile. Estimates of the sap tensions around which most cavitation in the leaf occurs can be made by finding the sap tensions at which click frequency is maximal. However, this method cannot be used to discriminate between a low click frequency caused by near exhaustion of the ability of the sample to produce clicks and the

effects of a slow rate of water loss. As a survey technique for finding the sap tension at which cavitation begins or is most likely, this technique is useful.

Table 8. Characteristic sap tensions of cavitating leaves of different species and populations of eight species of herbs, shrubs and trees. The methods by which the values have been calculated are as described in the text of sections 3.5.1 and 3.5.3. Values are ± S.E.

Species	a. Maximum click frequency	b. at which fraction of total clicks occur 10%	50%	c. Tension increment with most clicks	d. As (c) but from averaged profile
Acer (F)	1.72 ± .03	1.35 ± .05	1.70 ± .03	1.78 ± .00	1.8 (n = 10)
Acer (G)	1.59 ± .52	1.33 ± .08	1.57 ± .03	1.60 ± .00	1.6 (n = 7)
Alnus (F)	1.95 ± .10	1.72 ± .22	2.07 ± .02	2.00 ± .07	2.0 (n = 11)
Eucalyptus (G)	2.52 ± .06	1.71 ± .03	2.19 ± .04	2.59 ± .07	2.6 (n = 19)
Fraxinus (F)	2.00 ± .50	1.92 ± .14	2.89 ± .09	2.84 ± .02	3.4 (n = 10)
Lycopersicum (G)	0.15 ± .04	0.13 ± .01	0.30 ± .02	0.21 ± .04	0.25 (n = 9)
Plantago (F)	1.59 ± .07	1.27 ± .13	1.62 ± 0.9	1.68 ± .09	1.8 (n = 6)
Plantago (G)	1.73 ± .02	1.58 ± .08	1.81 ± .05	1.73 ± .05	1.8 (n - 13)
Rhododendron (F) (July 1981)	1.78 ± .10	1.59 ± .06	2.00 ± .06	2.09 ± .08	2.2 (n = 12)
Ricinus (G)	0.37 ± .18	0.36 ± .02	0.67 ± .04	0.76 ± .06	0.6 (n = 13)

Abbreviations : F - Leaves collected from plants growing in the field.

G - Leaves collected from plants growing in the glasshouse (section 2.1).

Table 9. Characteristic sap tensions of cavitating Rhododendron leaves sampled at intervals over two years. Methods and abbreviations are as for table 8. Temperatures are monthly means (sum of daily minimum and maximum divided by two). Temperatures were measured at Glasgow Airport.

	Characteristic Sap Tensions (MPa S.E.)					Temperatures	
Sampling Date	a. Maximum click frequency	b. Fraction of total clicks 10%	b. Fraction of total clicks 50%	c. Increment with most clicks	d. Averaged profile with most clicks	Monthly mean	Monthly minimum
December 1979	1.48 ± .08	0.69 ± .06	1.27 ± .03	1.45 ± 0.20	1.6 (n = 5)	3.8	- 5.6
January 1980	ND	1.31 ± .11	0.93 ± .11	2.4 ± 0.35	2.2 (n = 4)	1.7	- 9.4
April 1980	2.26 ± .21	1.41 ± .08	2.02 ± .06	2.18 ± 0.10	2.2 (n = 9)	8.7	- 1.7
June 1980	1.35 ± .05	1.24 ± .05	1.82 ± .07	2.12 ± 0.13	1.9 (n = 8)	12.9	5.0
October 1980	1.21 ± .55	1.05 ± .06	1.62 ± .05	1.7 ± 0.10	2.0 (n = 14)	8.1	5.0
January 1981	1.46 ± .06	1.24 ± .01	1.74 ± .05	1.68 ± 0.38	2.0 (n = 10)	4.5	- 5.6
April 1981	1.66 ± .10	1.16 ± .18	1.97 ± .06	2.12 ± 0.08	2.1 (n = 12)	7.9	- 4.4
July 1981	1.78 ± .10	1.59 ± .06	2.00 ± .06	2.09 ± 0.08	2.2 (n = 12)	14.3	4.4
Immature leaves (July 1981)	0.72 ± .03	0.69 ± .01	0.9 ± .31	0.80 ± 0.00	0.8 (n = 4)	14.3	4.4

3.5.4. Cavitation during controlled development of sap tension

The results of experiments designed to separate the effects of sap tension and loss of turgor in causing clicks are shown in figure 31.

As discussed in sections 2.2 and 3.2, clicks detected by the acoustic detector occur in conjunction with other, 'tissue' noises. Clicks have also been reported to occur when osmotic solutions supplied to the xylem cause the leaf to wilt without the development of sap tensions (Milburn, 1973a). An inspection of figure 30 also shows that the sap tensions at which clicks are most frequent are often close to those at which incipient plasmolysis (Ψ_p', section 3.4.4) occurs.

The possibility that clicks and cell turgor might be related, and in particular that clicks might be noises produced by cells pulling apart as they lose turgor, clearly needs investigation.

The loss of cell turgor and the development of sap tension were separated using the pressure chamber.

Cell turgor was reduced to zero by overpressurising the leaf to force water from the cells. After equilibration at the desired pressure, sap tension can be imposed in a controlled manner by lowering the pressure in the chamber (Tyree and Hammel, 1972).

Sap tension can then be calculated from the difference between the balance pressure of the leaf after equilibration and the chamber pressure.

Rhododendron leaves were used for these experiments because a) the sap tensions at which click production is maximal and those at which turgor is reduced to zero are close in leaves of this species, and b) leaves of this species have desirable mechanical properties (loud clicks, tough, crush resistant petiole, and are small enough to fit into the pressure chamber without touching the sides).

A turgid Rhododendron leaf was mounted in the pressure chamber and an acoustic detector mounted in the petiole as shown in figure 3. The experiments were conducted outside the sound-proofed cabinet normally used for acoustic experiments. To reduce noise to acceptable levels the pressure chamber was

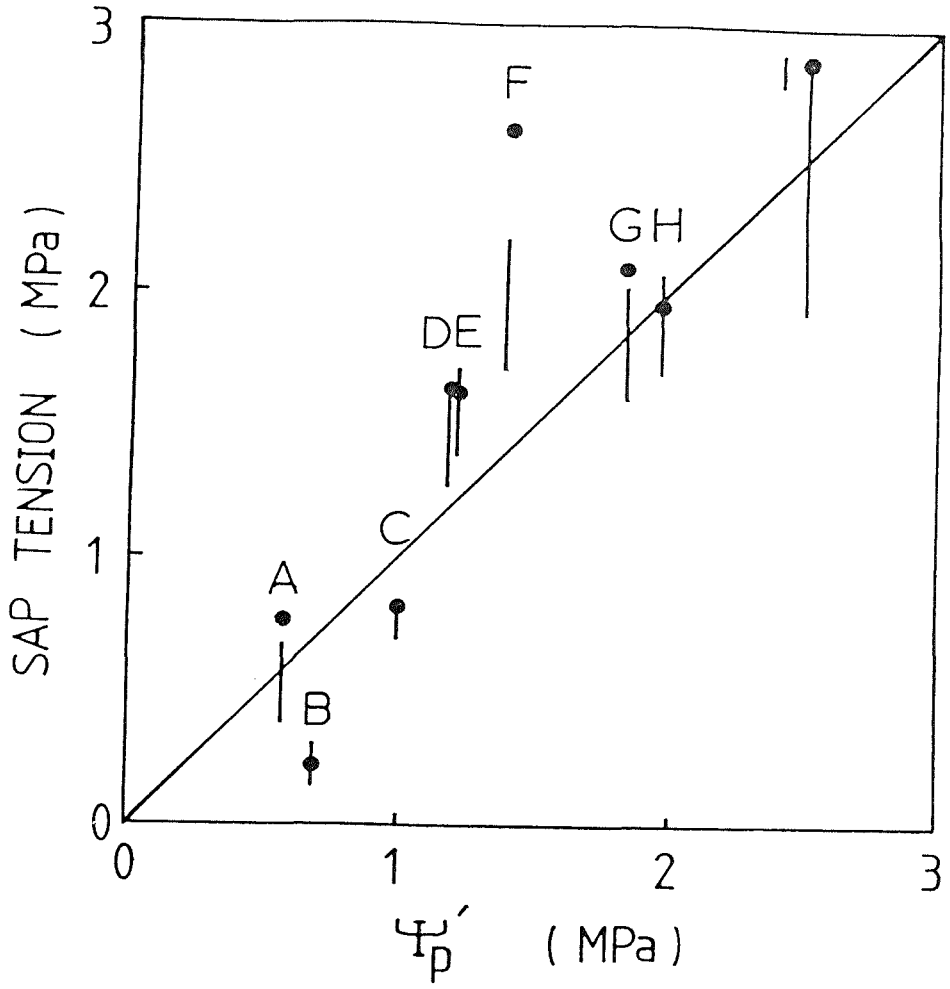


Figure 30. The relationship of the sap tensions at which cavitation occurs to the water potential of the leaf at incipient plasmolysis. Both the sap tension at which the cavitation profiles of each species 'peak' (•) and the range of sap tensions between which 10% and 50% of the clicks are detected (———) are shown.

A = Ricinus, B = Lycopersicum, C = Rhododendron (immature leaves),
D = Plantago, E = Acer, F = Eucalyptus, G = Rhododendron (July 1981),
H = Alnus, I = Fraxinus.

supported on layered piles of cotton wool and fibre matting and the acoustic detector supported on another pad of cotton wool.

It was found that clicks could only be recorded automatically as chamber pressure fell. Bubbling of sap on the end of the petiole as pressure was increased caused so much noise that clicks were undetectable above the background noise. With great care sap could be removed from the petiole as it was expressed, lowering noise levels sufficiently for clicks to be monitored aurally. Click-like sounds were heard as chamber pressure increased, but were very few compared to the many heard as pressure fell.

A typical result of experiments in which previously turgid Rhododendron leaves had been equilibrated at high chamber pressure and click production monitored as chamber pressure was released are shown in figure 31a.

Few clicks were detected until sap tension was slightly in excess of 2 MPa after which the frequency of clicking rose sharply. Click frequency declined as sap tension rose above 3.5 MPa (figure 31a). A burst of 'clicks' occurring over the last 1 MPa increase in sap tension was due to noise caused by visible movements of the rubber bung sealing the leaf into the pressure chamber.

Results similar to those shown in figure 31a were found in eight other experiments in which the leaf had been equilibrated at balance pressures in excess of the sap tensions known to cause clicks to be produced in leaves on the acoustic detector.

Equilibration at pressures lower than those causing cavitation

The results of an experiment in which the leaf was first equilibrated at a chamber pressure below the sap tension at which clicks are first detected in Rhododendron leaves (about 1.2 MPa, table 9) and the pressure slowly released, are shown in figure 31b. Except for the brief burst of noise caused by movement of the rubber bung as the last of the pressure was released, clicks could not be detected above the background noise.

When the same leaf was then equilibrated at a pressure equivalent to

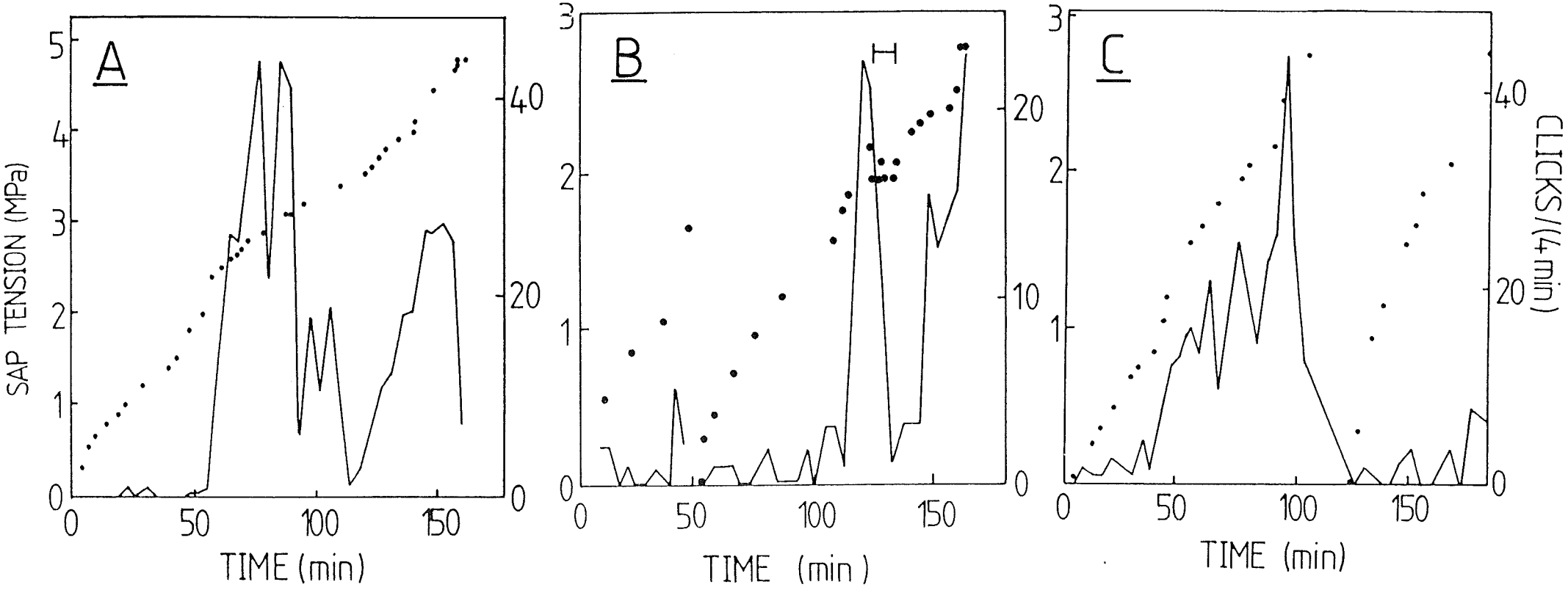


Figure 31. The occurrence of clicks when sap tensions are controlled using the pressure chamber.

A. Single cycle up to pressure greater than the sap tension causing cavitation. The noise over the last minutes of the experiments is an artifact.

B. Two pressure cycles, the first to less than cavitating sap tensions, the second to greater. The fall of pressure on the second cycle was halted for a short time as indicated (H).

C. Two pressure cycles, both to greater than cavitating sap tensions.

greater than the sap tension at which cavitation occurred clicks were produced as sap tensions rose above 1.5 MPa. This experiment was repeated three times with similar results in each case.

Interrupting the rise in sap tension when clicking is established

Also shown in figure 31b is the effect on click rate of temporarily stopping the rise in sap tension by halting the decline in chamber pressure. Click frequency fell immediately the fall in chamber pressure was halted and resumed quickly when pressure started to fall again. Noise due to movement of the rubber bung is apparent over the last 8-10 minutes of the experiment.

The rapid response of click frequency to a halt in the decrease of chamber pressure indicated, by reasoning analogous to that for the effect of changes in transpiration rate on click frequency (section 3.2.3), that xylem water potential and chamber pressures were close to equilibrium.

Click production on a second cycle to greater than cavitating sap tension

In figure 31c are shown the results of an experiment in which a Rhododendron leaf was taken through two pressure cycles, both resulting in sap tension greater than those required to induce cavitation. Clicking is clearly evident as sap tension rises above 1 MPa on the first pressure cycle, but is entirely absent on the second cycle.

Calculation of cavitation profiles

Figure 32 shows cavitation profiles calculated from pressure chamber experiments (including those shown in figures 32 and 34) which resulted in greater than cavitating sap tensions being produced in leaves. The last few minutes of the experiment were left out of the calculations to exclude noises caused by movement of the rubber bung.

For clarity only five of nine profiles are shown as the profiles obtained from pressure chamber experiments were more variable than those obtained from

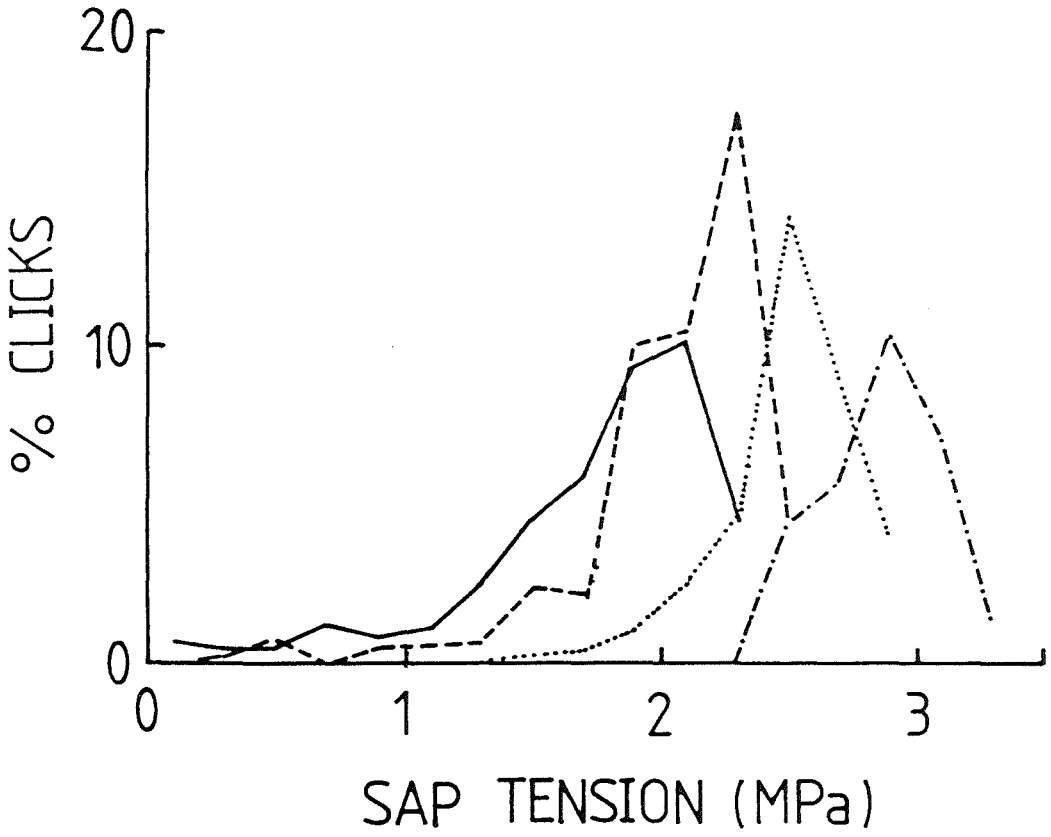


Figure 32. Cavitation profiles of Rhododendron leaves derived from experiments in which changes in sap tension were controlled using the pressure chamber.

The results of experiments with four different leaves (indicated by different lines) are shown.

experiments conducted in the usual manner (e.g. figure 27). The sap tensions at which the 'peak' occurred in individual experiments are given in table 10. The mean value of the sap tension at which the 'peak' of these cavitation

Table 10. Sap tension at midpoint of the 0.2 MPa increment in sap tension over which the greatest proportion of clicks occurs in acoustic experiments in which sap tension was controlled using the pressure chamber.

Experiment	Sap tension at 'peak' of cavitation profile (MPa)
1	2.4
2	2.4
3	1.4
4	2.2
5	1.9
6	2.8
7	3.2
8	2.0
9	2.3
$\bar{x}$ ± S.E.	2.29 ± 0.17

profiles occurs should be compared with the value of the same parameter obtained from experiments of the normal type conducted using leaves sampled at about the same time of year (July 1981), i.e. 2.09 ± 0.08 MPa (table 9).

Changes in leaf balance pressure during experiments

As can be seen in figure 31, the sap tension calculated for a second pressure cycle was less than that on the first. The difference was due to a decrease in leaf balance pressure measured after chamber pressure had been

released to zero in the first pressure cycle. Differences between balance pressures at the end of the equilibration period and after pressure has been slowly reduced to zero during the first cycle are given for four of the experiments in table 11.

Table 11. Chamber overpressure and a) leaf balance pressure after 30-60 min equilibration at this pressure, and b) leaf balance pressure after pressure had been slowly reduced to zero while click production was monitored.

Experiment number	Overpressure applied (MPa)	Balance pressure after equilibration (MPa)	Balance pressure after chamber pressure reduced to zero (MPa)	Difference between first and second balance pressures (MPa)
1	1.9	1.8	1.6	0.2
2	3.0	2.92	2.85	0.07
4	2.07	1.81	1.59	0.22
8	4.14	3.31	2.75	0.56

Three explanations of this decline in balance pressure were considered:

i) that equilibration of Ψ_1 with chamber pressure at high pressures had been incomplete.

ii) that Ψ_1 had changed during the experiment as a result of cell damage.

iii) that xylem conduits embolised as sap tension increased did not refill when the second balance pressure measurement was made so that less sap needed to be expressed from cells to return sap to the end of the petiole.

i) Errors in balance pressure determinations

This was thought to be the most likely cause of error in calculation of sap tensions.

Errors in measurement of the balance pressure after equilibration at high chamber pressure were considered to be more likely than were errors in balance pressures measured later when chamber pressure had fallen slowly to atmospheric. This was despite the apparently constant balance pressures over several minutes after 30-60 min equilibration at high pressure. Studies of the kinetics of sap expression from leaves (section 4.5) had shown that leaves were slow to attain a new equilibrium after imposition of a large pressure increment. Moreover, Jones and Higgs (1980) found that the balance pressures of leaves which had lost water by transpiration were lower than those from which water had been forced by overpressure in the pressure chamber. They also attributed the difference between balance pressures to inequilibration of Ψ_l with chamber pressure after a large pressure increment had been applied.

It was unlikely that the second measurement of leaf balance pressure was erroneously low as experiments had shown that balance pressure measurements of Rhododendron leaves were not subject to misequilibration errors if pressure was raised at .005 MPa s^{-1}, as was the case in this instance (section 3.3.2).

ii) Changes in Ψ_l of leaves in the pressure chamber

Leaves may have been damaged by long periods at high pressure in a nitrogen atmosphere. However, experiments had shown that the balance pressures of similar leaves held at high air pressures (and hence at high partial pressure of nitrogen which comprises 70% of the air) were unchanged (section 3.3.2). Alternatively, the cells may have become anaerobic in the pure nitrogen atmosphere (Tyree et al., 1978). Cell damage, as shown by increased leakage of solutes to the xylem sap (figure 22), may also have occurred as a result of reduced leaf water status alone (Scherbakova and Kasperska-Palacz, 1980; Leopold et al., 1981).

iii) Changes in xylem volume

The third possibility considered was that conduits embolised by cavitation did not refill when the second balance pressure measurement was made, so that less sap needed to be expressed from cells to return sap to the end of the petiole (Slavik, 1974). However, the results of experiments comparing the balance pressures of leaves and shoots showed that such conduits probably would refill on a second balance pressure measurement. In addition, cavitation had little capacity to affect balance pressure measurements of sap tension in leaves (section 3.4).

Discussion

Cavitation profiles drawn from pressure chamber experiments peaked at sap tensions around 2.3 MPa. This compares favourably with the peak at 2.1 MPa (table 9) of profiles drawn from the data of experiments in which sap tensions increased as the leaves transpired. However, the cavitation profiles of pressure chamber experiments were more variable in shape and the sap tensions at which they peaked than were those from experiments in which the leaf lost water by transpiration.

Few clicks were detected as the cells of the leaf lost turgor. However, many clicks were detected when, through a decline in chamber pressure, sap tensions had increased to magnitudes similar to those at which clicks began in transpiring leaves.

Therefore, it seems that clicks are produced when a critical sap tension is achieved and are not the result of loss of cell turgor.

The assertion by Milburn (1973a) that clicks detected in Ricinus leaves wilted by infusion of solutions of low osmotic potential were not caused by loss of cell turgor therefore seems to be justified.

The cause of the few clicks heard as chamber pressure increased is unknown. They may have been due to movement of the pressure chamber seal as pressure increased, or to compression of leaf tissues by high gas pressures.

The greater variability in the cavitation profiles obtained from pressure chamber experiments compared to those from normal acoustic experiments was thought to be an artifact resulting from errors in the calculation of sap tensions. These errors were apparently caused by incorrect measurements of balance pressure after equilibration of the leaf at high pressure. They were not due to large inequilibria between sap tension and the falling chamber pressure during experiments.

These errors in balance pressure measurements will result in over-estimates of the sap tensions at which cavitation occurs. However, as the inequilibria which are probably responsible for the overestimation disappear slowly over the entire duration of the experiment, it is not possible to correct the results by substituting the second balance pressure measurement when calculating sap tensions.

3.6. Recovery from cavitation

3.6.1. Introduction

The effect of cavitation on plant water status depends not only on how many and which conduits cavitate, but also on for how long the conduits remain embolised.

If embolisms exist for only a short period before collapsing it is possible that even extensive cavitation will have little lasting effect on the ability of the xylem to supply the water requirements of the plant (Milburn and McLaughlin, 1974).

Recovery of water conduction in a cavitated plant can take two forms:

a) If emboli are not redissolved the capacity to conduct water can be recovered only by laying down new xylem conduits, as happens every spring in some deciduous ring-porous trees such as Acer (Kozlowski and Winget, 1963).

b) If emboli are dissolved, the conduction of water in the xylem can be restored without producing new xylem conduits.

The acoustic technique provides a useful means of testing for removal of emboli from xylem conduits. This is because the clicks detected by the acoustic detector are produced when strain in conduit walls is released by cavitation of the contained sap. If the conduit contains a bubble the sap cannot come under tension as the bubble will expand. Therefore the walls of the conduits will not deform and store the energy which produces the 'click' detected when sap tension is released by cavitation.

The recovery of the ability of leaves to produce clicks and, by the above argument, to sustain cohesive sap transport has been investigated in leaves of Lycopersicum (Nonhebel, pers. comm.), Plantago (Milburn and McLaughlin, 1974) and Ricinus (Milburn, 1973a).

In these studies the ability of leaves, which had almost ceased to produce clicks in one drying cycle, to produce clicks could be partly recovered by supplying water at atmospheric pressure to the xylem for several hours. The recovery was enhanced if water was supplied under positive pressure or if the gas pressure in the embolised conduits was lowered before supplying water.

Information on the recovery of sap columns in intact plants from cavitation is lacking although it has been suggested that cavitation and recovery, aided by root pressure, may be a common diurnal phenomenon in some herbs (Milburn and McLaughlin, 1974).

In the experiments described below the recovery of sap column continuity in cavitated leaves, shoots and whole plants was studied in Rhododendron by using the acoustic technique.

It had already been established (section 3.5) that cavitation, as indicated by the acoustic technique, occurred at sap tensions of 1.2-3.0 MPa in both leaves and shoots of Rhododendron

3.6.2. Recovery of clicks in Rhododendron by rehydration

Obtaining leaves of known sap tension

When investigating recovery from cavitation it was necessary to be able to assess the sap tensions in leaves without taking balance pressure measurements of the leaves themselves as some cavitation emboli might dissolve during balance pressure measurements. The experimental approach adopted was to measure balance pressures and assess recovery from cavitation on different leaves from the same shoot.

Shoots were allowed to develop water stress by transpiration on the laboratory bench before sealing into plastic bags to equilibrate for thirty minutes. After equilibration groups of leaves were cut from the stem and their balance pressures determined simultaneously by mounting them together in the split-bung seal of the pressure chamber.

Variation in balance pressure between leaves sampled at mean balance pressures below 1.5 MPa were very small and of magnitudes comparable to the accuracy with which pressure could be read from the pressure gauge (about ± 0.01 MPa). Variability within groups increased as mean balance pressure increased above 1.5 MPa. When mean balance pressure had risen to 2.7 MPa the balance pressures of some leaves within the group were found to be as much as 0.25 MPa from the group mean.

This degree of variability was acceptable since, for the purposes of these experiments, it was sufficient to know whether or not sap tensions in the leaves were in excess of those required to cause cavitation.

In rehydration experiments variations in sap tension between leaves used for acoustic experiments and for balance pressure measurements were minimised by using adjacent leaves. This precaution was especially important when the rehydration of shoots was being studied. Variation between individual leaves from a rehydrated shoot was greater than between leaves taken from the same shoot before rehydration. This aspect is discussed further in section 4.5.

a) Rehydration of leaves

Figure 33 shows the results of experiments in which the recovery of the continuity of sap columns in cavitated leaves supplied with distilled water at atmospheric pressure was monitored using the acoustic technique.

Leaves were cut under water and set to rehydrate in a humid atmosphere with their petioles in distilled water. At intervals leaves were removed in pairs and one placed on the acoustic detector to monitor clicking as the leaf dried and the other used for balance pressure determination. The leaves were then discarded.

Figure 33 A and B are records of clicks produced by leaves from shoots of leaf balance pressure 0 and 1.22 MPa respectively without an intervening period of water uptake after excision. In both cases the patterns of click production are very similar to those described for uncavitated leaves in section 3.2.2.

Figure 33C shows data from a leaf sampled from a shoot of leaf balance pressure 3.15 MPa and therefore expected to be almost fully cavitated. The inability to detect clicks above the background noise supports this supposition.

Figure 33D. Leaf removed from a shoot of balance pressure 3.15 MPa and rehydrated for two hours. A few clicks occurred in a small peak. Leaves hydrated for a similar period had balance pressure of 0.05-0.10 MPa. After four hours rehydration (figure 33E) clicks are detected in a pattern similar to that of uncavitated leaves although the number of clicks detected is lower. Balance pressure of similar leaves was 0.0-0.05 MPa.

After 16 and 43 hours rehydration (figure 33 F and G) respectively clicks occur, but a longer initial delay was found (cf. figure 7).

None of the leaves in the above experiments showed discoloration or brittle areas on the lamina, suggesting that the leaves had not been seriously harmed by the water stress to which they had been subjected.

Results very similar to those described above were found in two other trials.

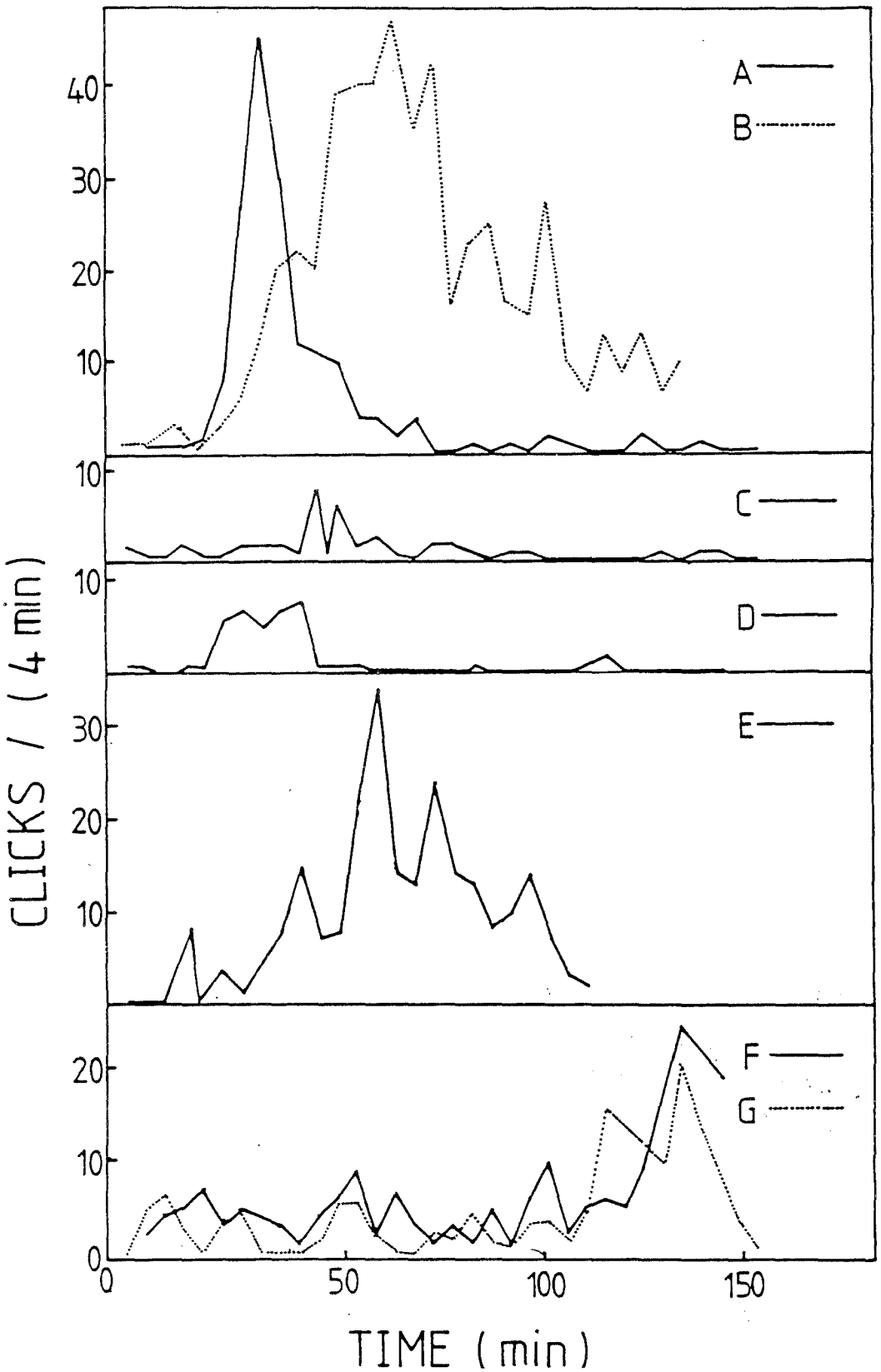


Figure 33. The recovery of click production in cavitated leaves rehydrated after excision from the shoot.

A = Turgid, leaf balance pressure = 0-0.05 MPa.

B = Stressed, but not cavitated leaf, leaf balance pressure = 1.22 MPa.

C = 'Cavitated' (maximum balance pressure = 3.15 MPa).

D = 'Cavitated' (as in B), rehydrated 2 hours, balance pressure = 0.05-0.1 MPa.

E = 'Cavitated' (as in B), rehydrated 4 hours, " " = 0 -0.05 MPa.

F = 'Cavitated' (as in B), rehydrated 17 hours, " " = 0 -0.05 MPa.

G = 'Cavitated" (as in B), rehydrated 43 hours, " " = 0 -0.05 MPa.

b) Rehydration of shoots

The results of an experiment investigating the recovery of sap columns in leaves attached to 150-200 mm long cavitated Rhododendron shoots are presented in figure 34. The experiment was conducted four times in all, with a different shoot on each occasion. Results in all four experiments were similar to those shown in figure 34.

A shoot was stressed to greater than cavitating sap tension and equilibrated in a sealed plastic bag overnight. Leaves were then sampled for measurement of balance pressure and the ability to produce clicks. 40mm of the stem was then trimmed under water to remove emboli and the stem set in a humid atmosphere with its end in distilled water to rehydrate. At intervals pairs of adjacent leaves were cut and their balance pressures and ability to produce clicks determined.

Figure 34A. Clicks produced by a leaf cut from the shoot after stressing to subcavitating tension (leaf balance pressure 1.31 MPa). The pattern of clicks produced is typical of that for uncavitated leaves (section 3.2.2).

Figure 34 B and C. Leaves sampled from the shoot stressed to leaf balance pressure of 3.10 MPa ; before rehydration (B) or after three and a half hours of rehydration (leaf balance pressure 2.63 MPa) (C). No clicks could be detected above the background noise in either case.

Figure 34D. Leaves from the same shoot after rehydration for twenty-two hours (leaf balance pressure 0.0-0.05 MPa). The pattern of click generation was similar to that in previously uncavitated leaves although the initial increase in click frequency occurred later and was more drawn out than usual for unstressed leaves.

The same procedure as that described above was used later in the project when investigating the effect of cavitation on sap transport. In none of these experiments could clicks be detected in leaves which had not recovered their balance pressures to less than 0.05 MPa after a period at cavitating

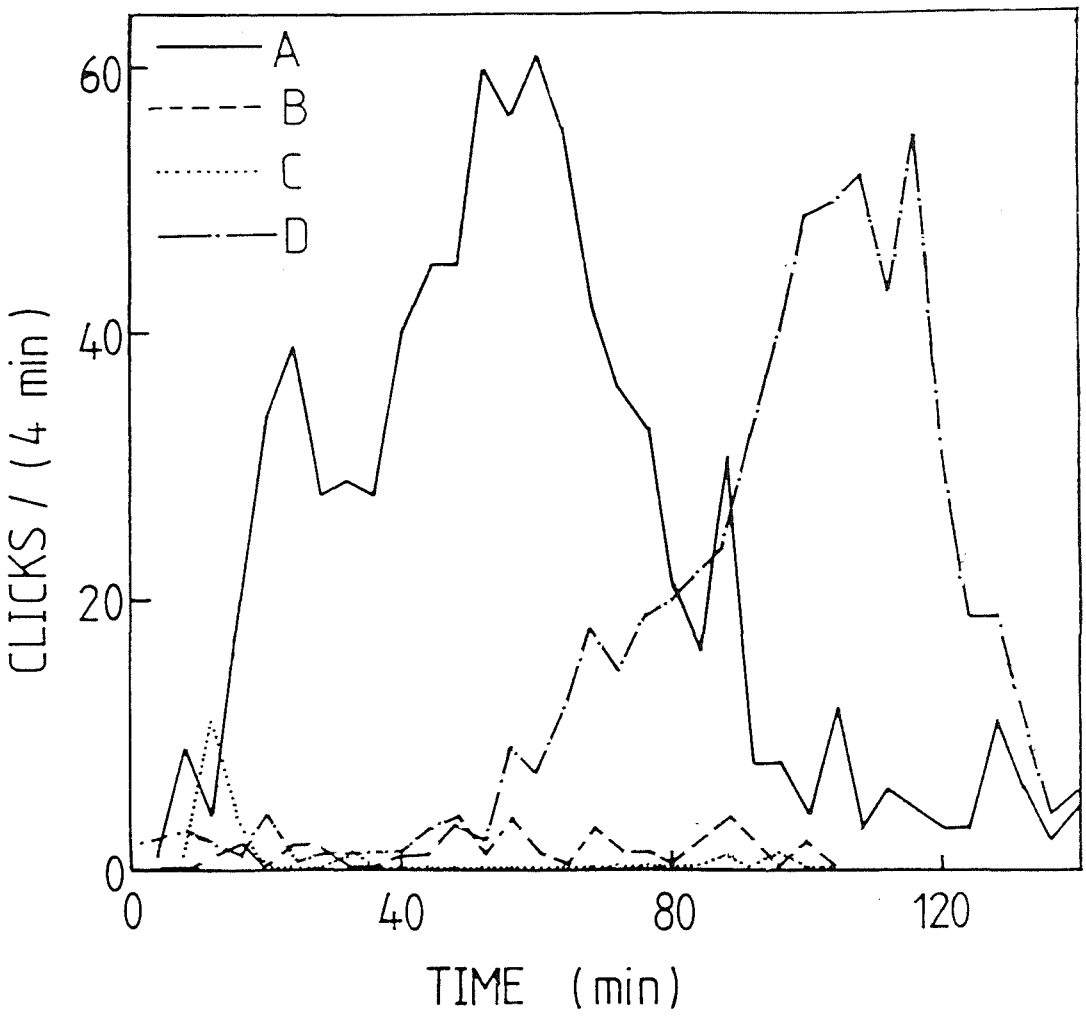


Figure 34. The recovery of click production in leaves attached to shoots.
Leaf from:

A - Uncavitated but stressed shoot. Balance pressure = 1.31 MPa.
B - 'Cavitated' shoot. " " = 3.19 MPa.
C - " " rehydrated 3.5 hours. " " = 2.63 MPa.
D - " " " 22 hours " " = 0-0.05 MPa.

sap tension.

c) Rehydration of plants

In figure 35 are presented the results of experiments in which the recovery of leaves on whole plants from cavitating water stress was investigated.

Potted Rhododendron plants were subjected to increasing water stress over a five week period in June and July 1982. Recovery from cavitation was assessed when the plants were watered twice daily to keep the soil in the pots at near field capacity. Experiments were carried out in duplicate. Results were similar in both cases.

Figure 35A. Clicks produced by a leaf from a plant on the normal glasshouse watering cycle (maximum daily balance pressure 0.5-0.6 MPa; chapter 4). Click frequency rose very quickly and as quickly fell again. This was followed by a long period over which the low click frequency declined still further.

Figure 35B. Leaf from moderately stressed plant (leaf balance pressure 1.66 MPa; diurnal changes in balance pressure were almost absent at this level of stress (section 4.6). After an initial delay click frequency peaked in a similar way to that in experiments with uncavitated leaves (section 3.2.2).

Figure 35C. Leaf from a severely stressed plant (leaf balance pressure 2.37 MPa). Except for a brief burst of clicks or noise immediately after mounting on the acoustic detector clicks could not be detected above the background noise.

Figure 35 D and E. Leaves from a plant subjected to severe water stress (maximum leaf balance pressure attained was 2.33 MPa) and rehydrated for (D) two days (maximum leaf balance pressure over the day that the leaf was sampled was 0.3 MPa), or (C) seven days (maximum daily balance pressure 0.55 MPa). No clicks were detected in either case.

Clicks in last expanded leaves

Clicks were difficult to detect in the mature leaves (about one year old) used for the experiments with potted plants described above (figure 35).

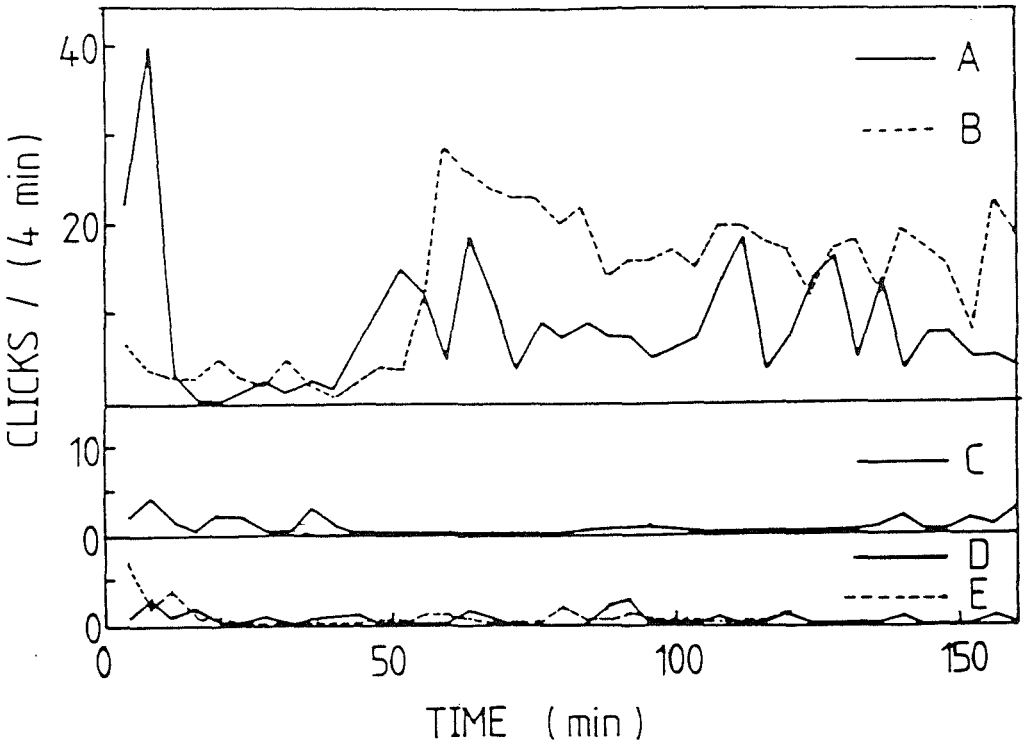


Figure 35. The recovery of click production in leaves attached to intact plants.

Leaf from:

A - Plant on normal watering cycle. Maximum balance pressure = 0.5-0.6 MPa.

B - Moderately stressed plant. Balance pressure = 1.66 MPa.

C - 'Cavitated' plant. " " = 2.37 MPa.

Severely stressed plant (balance Pressure = 2.33 MPa) which had then been:

D - Rewatered for 2 days. Maximum daily balance pressure = 0.3 MPa.

E - Rewatered for 7 days. " " " " = 0.55 MPa.

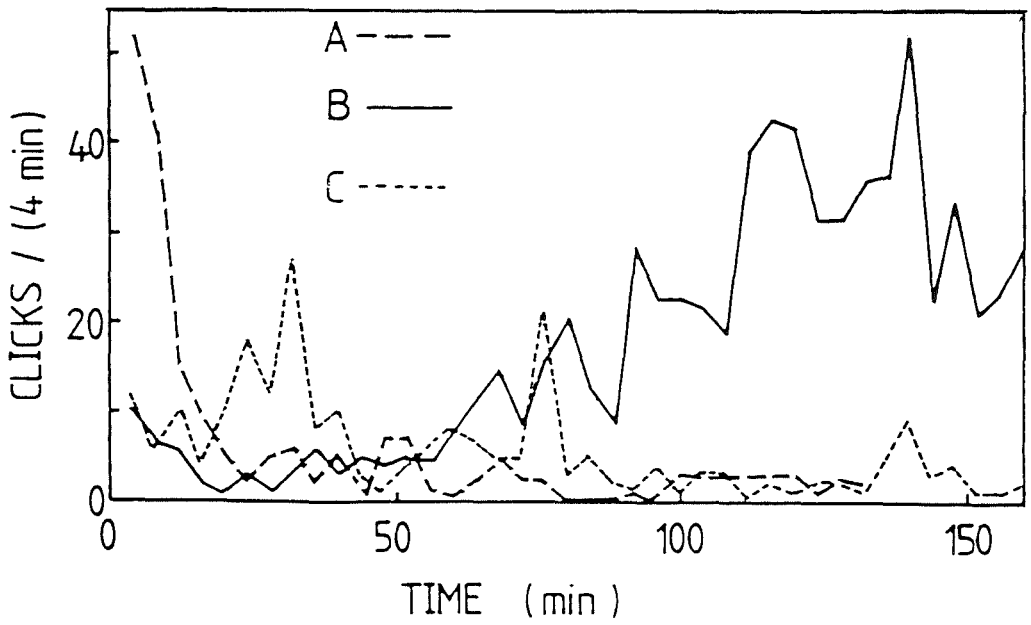


Figure 36. Click production by recently expanded leaves from plants in the glas shouse.

A - Recently expanded leaf from a well water plant.

B - " " " " a stressed plant. Balance pressure = 2.16MPa.

C - " " " " " " " " " " = 2.28MPa.

These leaves were smaller and softer than those which had expanded in the three months prior to these experiments. Clicks could be more easily detected in the younger leaves than in the older. Because there were comparatively few recently expanded leaves on the plants, the older leaves were used for most of the experiments.

Typical results of experiments using these younger leaves are shown in figure 36. The pattern of clicks detected in the last expanded leaves from a well watered plant (fugure 36A) was similar to that found in older leaves from similar plants (figure 35A).

When the plants were water-stressed (balance pressures 2.16 and 2.28 MPa, figures 36B and C respectively) there was a longer delay between mounting on the detector and the detection of the first clicks than in leaves from 36A.

3.6.3. Discussion

a) Recovery of clicks

The ability to detect clicks in leaves which had been subjected to severe water stress was recovered when water at atmospheric pressure was made available to the xylem of either the water-stressed leaves or the shoot to which they were attached.

Recovery was not immediate. Moreover, recovery took longer if the leaves were left attached to shoots than if detached leaves were supplied with water. In leaves, the ability to produce clicks was partly recovered after two hours of hydration and almost fully recovered after four hours. In shoots little or no recovery was found after three and a half hours rehydration but recovery appeared complete after twenty-two hours.

Comparison of the balance pressures of leaves (or leaves from shoots) rehydrated for similar periods showed that clicks were not recovered until the leaf balance pressure had fallen below 0.05 MPa. As this was near the lower limit to which chamber pressure could be read accurately from the Bourdon gauge, the actual balance pressure in leaves before reconstitution of sap columns may

have been less. Hydrostatic pressure in the xylem sap of a leaf of balance pressure 0.05 MPa is approximately 0.5 atmospheres, and increases to atmospheric as balance pressure falls to zero. Recovery of the ability to produce clicks was apparently completely inhibited by any tension in the sap; that is, if sap Ψ_p is less than the vapour pressure of the sap (.0032 MPa if the sap is water).

Clicks were apparently not recovered in leaves on whole plants which had been subjected to cavitating water stress and which had subsequently been well supplied with water. It was known (section 4.6) that these well-watered plants were still subject to daily leaf balance pressure maxima of 0.3 MPa or more, and that minimum balance pressures during the night were 0.03-0.04 MPa.

The failure to detect clicks in leaves from stressed and rewatered plants was therefore consistent with the observations concerning recovery and residual sap tensions in leaves and shoots.

Observations of the behaviour of bubbles in the xylem of some plants revealed that the bubbles expanded if the plants were water-stressed but shrank if the plant was well watered (review by Crafts et al., 1949). At the time that the experiments were conducted it was not possible to measure the turgor component of xylem sap water potential so that a quantitative relationship between sap pressure and the expansion or contraction of the bubbles could not be found.

Experiments using heat-killed stems of Impatiens (Dickson and Blackman, 1938) seem to confirm that the dissolving of bubbles in the xylem is almost certainly a purely physical process. Experiments by these workers also showed that increasing the difference between the gas concentrations in the bubble and the xylem sap (by boiling the sap to remove gas) increased the rate at which the bubbles dissolved. Moreover, bubbles dissolved faster in narrow than in wide xylem conduits, suggesting that capillarity was involved.

b) The physical basis of recovery from cavitation

If the bubble is to dissolve spontaneously the pressure inside the bubble, P_B, must be less than the pressure tending to compress it (Epstein and Plesset, 1950). The latter will be the sum of the hydrostatic pressure, P_H, and the capillary pressure, P_C, developed by surface tension at the meniscus around the bubble in the conduit.

Ideally (Eqn. 8) P_H = (atmospheric pressure - balance pressure) so that if the balance pressure is zero, as in a fully turgid leaf

$$P_H = (\ 0133 \text{ MPa} - 0)$$
$$= 0.1013 \text{MPa}.$$

P_C can be calculated from the formula for capillary rise,

i.e. Eqn 9. $$h = \frac{2\sigma \cos\theta}{r\rho g}$$ in which h = height of liquid column (m)

σ = surface tension of liquid (Pa m^{-1})

θ = wetting angle between liquid and capillary

r = radius of the capillary (m)

ρ = density of the liquid (kg. m^{-3})

and g = gravitational acceleration (m s^{-2})

By multiplying both sides of equation 9 by (ρg) the pressure generated by surface tension in the capillary (P_C) is obtained.

Eqn. 10. $$P_C = h\rho g = \frac{2\sigma \cos \theta}{r}$$

Assuming xylem sap to be water (σ = 72 x 10^{-3} Pa m^{-1}) and the xylem conduit walls to be fully wetted (θ = 0°), P_C is dependent on the capillary radius.

Radial diameters of vessels in a two year old stem of Rhododendron were measured and found to range between 20 and 40 µm. P_C in such capillaries will range from 0.0072 MPa to .0144 MPa.

If the bubble occupying the xylem conduit has recently formed by cavitation it will contain only water vapour at its vapour pressure (Huber, 1956),

i.e. P_B = 0.0032 MPa.

For a bubble in the narrowest xylem vessels

$r = 10\ \mu m$

therefore $P_C = .014$ MPa.

If the bubble is to collapse

$$P_H > (P_B - P_C)$$

so that $P_H > (.0032 - .014)$ MPa

$$P_H > -.0108 \text{ MPa.}$$

Therefore, by eqn. 8

Balance pressure < .0905 MPa .

Bubbles in wider conduits, in which P_C is lower, will collapse at lower maximum balance pressures than those in narrower conduits.

Diffusion of gases dissolved in the xylem sap into the bubble will raise P_B and restrict its collapse to lower balance pressures than if it contains only water vapour.

If the xylem sap is saturated with air

$$P_B = 0.1013 \text{ MPa}$$

and the bubble will not dissolve if sample balance pressure is greater than 0.014 MPa (eqn. 8).

If the sap is in equilibrium with gas pressure in the wood (which may be up to 0.2 MPa (MacDougal et al., 1929)) a P_H higher than atmospheric may be required to dissolve the bubble.

Conditions may be made more favourable for the collapse of bubbles in the xylem by lowering P_B (by using vacuum to extract gas from the embolus) or by increasing P_H (by root pressure or by use of the pressure chamber (Milburn 1973a, Milburn and McLaughlin, 1974).

c) Collapse of bubbles in xylem conduits

The longer time taken to restore clicks to leaves which are still attached to the shoot as compared to isolated leaves may have been due to the extra time taken to raise sap Ψ_p to near zero in the shoots. The shoots require more water to be taken up to alleviate water deficits (and so reduce sap tension)

than do leaves, but this water must be taken up through a long stem in addition to the leaf petioles. Friction in the xylem conduits, form drag at the pit membranes (Jeje and Zimmermann, 1979) and lateral transport of water to satisfy water deficits of the pith, xylem parenchyma, cambium and epidermis (e.g. Namken et al., 1971) will all combine to keep sap Ψ_p in the xylem of shoots low for extended periods. The kinetics of the rehydration of shoots and further evidence that xylem emboli do not dissolve until sap is at near atmospheric pressure are discussed in section 4.5.

d) Transpiration and click frequency

The short delay between the start of an acoustic experiment and the occurrence of the peak in click frequency in leaves which were taken from unstressed whole plants and used without rehydration was different to the response of leaves hydrated prior to experiments. The rapidity with which clicks occur in this situation has been attributed to pre-existing sap tensions in the plant which may be just below those required to cause cavitation, or which may already have resulted in partial cavitation before the leaf was excised (Milburn and McLaughlin, 1974).

It was known that sap tensions were of 0.3-0.6 MPa in the plants from which the leaves were cut (section 4.4). It was unlikely that cavitation occurred in these leaves at sap tensions below a minimum of about 1.2 MPa (section 3.5). It was therefore thought likely that the rapid rises in sap tension occurring in the several minutes between the time that the leaves were sampled and when they were mounted on the acoustic detector were responsible for the rapid onset of clicking. Very rapid changes in balance pressure immediately after leaf excision have also been reported in sorghum (Turner and Long, 1980).

A curious aspect of the results of these experiments (figures 33, 34, 35) is the tendency for the delay between the beginning of the experiment and the beginning of clicking to increase when the leaf or shoot has been subjected to a cycle of water stress and rehydration.

The longer delay could be the result of increased stomatal resistance in leaves which had been subject to water stress. Because of this delay, experiments had to be continued for longer than was normally necessary when using unstressed material. Failure to extend the duration of the experiments could have resulted in the conclusion that clicks were not recovered by rehydration.

This problem shows the limitations of the use of patterns of clicks against time to assess the recovery of plant material from cavitation. Without relating click frequency to changes in sap tension, or at least in water content, it is difficult to separate low click frequency due to the effects of cavitation from low click frequency due to slow increases in sap tension.

In discussing these experiments the occurrence of a peak in click frequency was taken to indicate recovery from cavitation. The number of clicks detected was considered to provide extra evidence of the extent of recovery but, for the reasons discussed in section 3.2, was considered to be less important.

Ideally, investigations into the recovery of clicks should aim to reproduce the cavitation profiles (section 3.5) of the leaf.

3.7. Summary of chapter 3

(1) The acoustic technique was successfully applied to the determination of the sap tension at which cavitation occurs in eight species of trees, shrubs and herbs.

Cavitation began at sap tensions of 1-1.5 MPa in leaves of Acer, Alnus, Eucalyptus, Fraxinus, Plantago and Rhododendron. Cavitation was

most frequent at sap tensions of 1.7-3 MPa in these species. Cavitation began at sap tensions of 0.1 MPa in Lycopersicum and Ricinus and was most frequent at sap tensions of 0.3-0.7 MPa.

Sap tensions at which cavitation occurred increased as leaves matured and, in Rhododendron, were unaffected by low winter temperatures.

(2) Dissolving of cavitation emboli appeared to be dependent on attaining near atmospheric pressures in xylem sap. Thus the ability to produce clicks could be recovered by rehydrating cavitated, detached Rhododendron shoots or leaves, but not by rewatering intact plants.

(3) Experiments showed that gas appeared in the xylem of Acer shoots at the same time that clicks were detected in the same shoot, and that the majority of clicks produced in Rhododendron leaves were produced as sap tension increased, not as cell turgor decreased.

(4) No evidence of errors in pressure chamber estimates of Ψ_l due to cavitation were found in Rhododendron leaves although differences between shoot and leaf balance pressures at sap tensions above 1.8 MPa may have been caused by cavitation in the xylem of the shoot stems.

Chapter 4. Cavitation and sap transport

4.1. Introduction

Cavitation of xylem sap results in the affected xylem conduits becoming gas filled and therefore unable to take part in cohesive sap transport. However the cellular construction of the xylem prevents the spread of cavitation emboli beyond the conduits in which they are formed and so reduces the effect of a single cavitation event on sap transport in the xylem as a whole (Dixon and Joly, 1894). The effect of cavitation on sap transport therefore depends on the degree of redundancy of conduits in the xylem as well as on the number of cavitation events. As yet no studies have investigated the effect of cavitation caused by known sap tensions on the flow of water in the xylem. The experiments described in this chapter were designed to measure the effects of cavitation (inferred from measurements of sap tension and knowledge of the sap tensions causing cavitation (section 3.5)) on the permeability of the xylem of Rhododendron ponticum. Ricinus communis was also used for some preliminary trials but, for reasons discussed below, was not used in large scale experiments.

The effects of high sap tensions on the permeability of the xylem of Rhododendron was investigated in i) segments of stem, ii) shoots and iii) whole plants. The three sets of experiments were necessary because, although measurement of the factors from which permeability can be calculated is easier and more accurate in smaller samples, there is an increasing tendency for cavitation emboli to dissolve as the sample size is decreased and, as resistances to water movement are also decreased, sap pressures rise. In whole plants and shoots sap pressure can only rise to atmospheric but in stem segments sap pressure will exceed this as water is supplied from a small hydrostatic head.

These experiments were undertaken specifically to investigate the flow of sap in water-stressed material. In this respect they are different to previous studies which have measured xylem permeability in unstressed material

(e.g. Farmer, 1918; Peel, 1965; Zimmermann, 1978) or material which had been thoroughly rehydrated before measurements were made (e.g. Edwards, 1980; Megraw, 1967; and Tyree and Zimmermann, 1971).

The effect of high sap tensions on the conduction of sap in the xylem was also investigated by using stains to identify the parts of the xylem in which sap was being carried. Dyes have been used previously to observe changes in sap conduction in trees in response to water stress (Greenidge, 1955a; Kozlowski and Winget, 1963) but the sap tensions causing these changes were not fully determined in these studies.

4.2. The vascular anatomy of Rhododendron and Ricinus

When designing or interpreting the results of permeability experiments it was necessary to know something of the arrangements of the tissues and the length of the xylem conduits in the samples used. Experiments measuring the length of the xylem conduits in stems of both Rhododendron and Ricinus were therefore conducted. The results of these experiments and a brief description of the anatomy of the stems of each species are given below.

Experiments with Rhododendron were conducted using the current season's shoots (sampled between January and July, and classified as 'one year old') or the previous season's growth ('two year old'). Similar experiments were conducted using 4-6 month old Ricinus plants grown in the glasshouse.

a) General anatomy

The Rhododendron shoots produced each year are 200-400 mm long and consist of a length of leafless stem with a cluster of leaves at the apical end. In cross-section the stems consist of a thin bark overlying a hollow cylinder of wood around a large central pith. The wood is composed solely of fibres and vessels (Rivett, 1920) and is frequently traversed by rays one or two cells wide. The leafless part of the stem tapers gradually towards the apical end.

Ricinus stems, consisting of the length between the roots and the first

leaf, were 0.4 to 1.0 m long. They consisted of a hollow cylinder of xylem overlaid by a thin bark and surrounding a very large pith, sometimes having a central pore.

b) Length of conduits in stems

One and two year old Rhododendron shoots and a Ricinus shoot were set to transpire diluted and filtered Indian ink suspension (section 2.8) overnight. At the end of this period leaves from the Rhododendron shoots had balance pressures between 0.6 and 1.0 MPa and the leaves on the Ricinus shoot had dried to brittleness. Both the elevated balance pressures of the Rhododendron leaves and the death of the Ricinus leaves were taken as evidence that the transport of water in the xylem had been blocked by ink drawn to the ends of the xylem conduits.

The Rhododendron stems were sectioned at 5mm intervals and the number of conduits containing ink in each section counted (figure 37a). An analysis of the results, analogous to that of Zimmermann and Jeje (1981), of all six stems showed that most xylem conduits terminated within 45-50 mm of the cut end of the stems and that less than 1% of the conduits extended more than 100mm from this end of the stem (figure 37b). Results were similar in three two year old shoots (both years' xylem) and in three one year old shoots.

Ink was found in vessels of the Ricinus stem as high as the first leaf. No other analysis of the results was carried out.

c) Length of conduits in leaves

Rhododendron leaves were set to transpire the Indian ink suspension overnight. On the basis of experience with shoots it was assumed that ink had been drawn to the ends of the conduits in the petioles by the end of this period. The petioles were sectioned 4mm from the cut end and at the base of the lamina (typically 8-12 mm from the cut surface) and the number of conduits containing ink in each section counted.

Ink was found only in the thin-walled, large diameter conduits (presumed

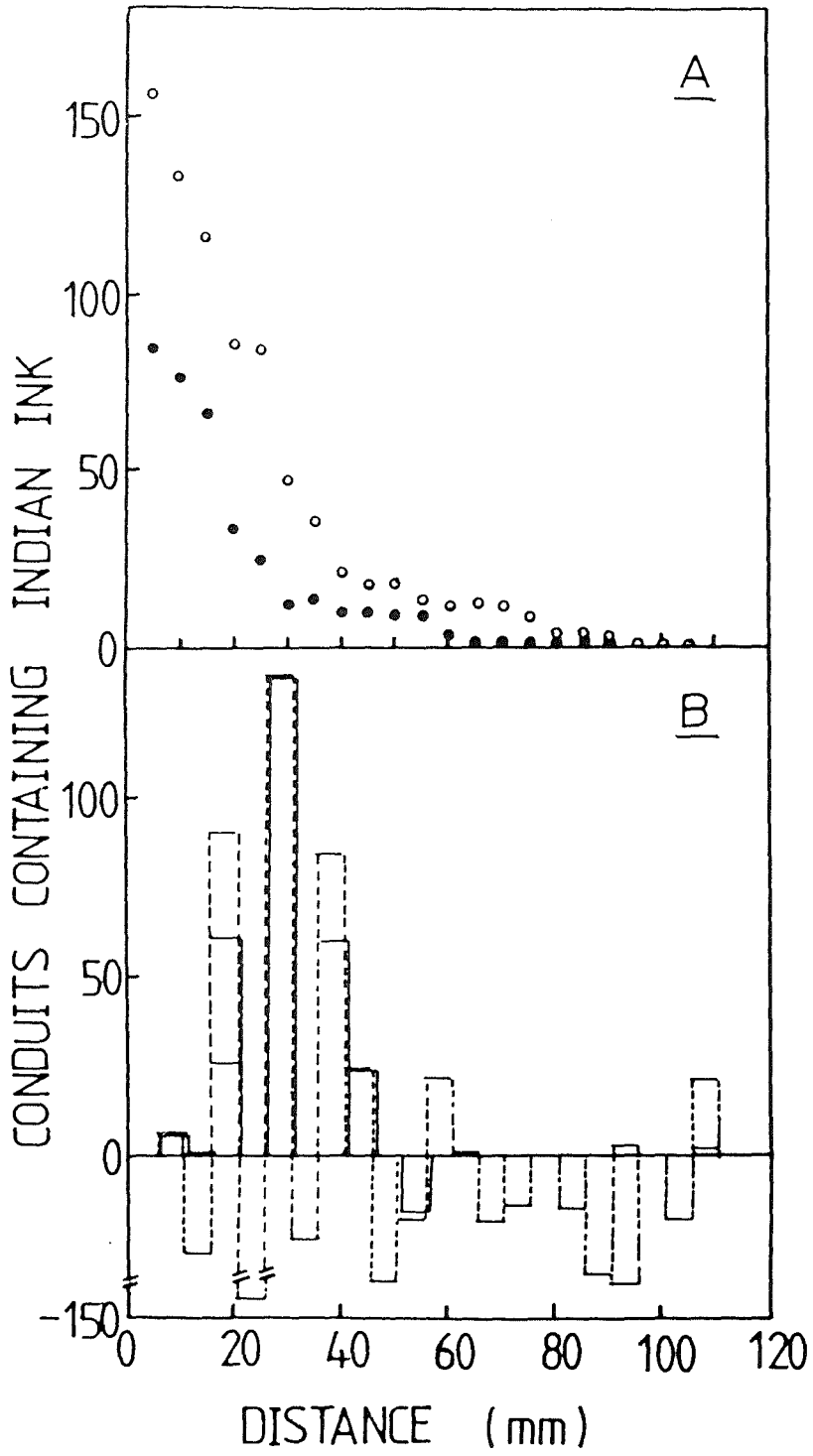


Figure 37. Determination of length of conduits in a two year old Rhododendron stem by injection of Indian ink.

A. Number of conduits containing ink at increasing distance from the injection surface. o - Outer growth ring. o - Inner growth ring.

B. Zimmermann and Jeje (1981) analysis of conduit lengths in the outer growth ring of the stem. The calculation of a negative number of conduits of a particular length (----) indicates non-random termination of conduits within the stem. The best estimates of the number of conduits of each length are shown (——).

to be vessels). 40-60% of conduits containing ink 4mm from the cut surface still contained ink at the base of the lamina (table 12).

Table 12. Number of conduits containing Indian ink in petioles of Rhododendron leaves injected after excision close to the stem.

Leaf number	Length of Petiole (mm)	Number of conduits containing ink at 4mm	laminal base
1	9	12	7
2	13	21	7
3	12	8	4
4	12	14	8
5	14	4	0
6	11	8	3

These experiments showed that most of the xylem conduits to which air had been admitted when Rhododendron stems had been water stressed after excision could be removed by trimming 40mm underwater from the ends of the stems. Trimming 50mm would remove almost all of the embolised conduits but would preclude using the same shoot in several experiments (section 4.5). Trimming 40mm from the stems was considered a reasonable compromise.

Because vessels extended over the whole length of Ricinus stems and Rhododendron leaf petioles all operations with these samples had to be conducted underwater to prevent air-emboli which could not be removed forming in the xylem.

4.3. The effect of cavitation on the paths of sap flow

The effect of cavitation on the paths of sap flow in Rhododendron stems was investigated by introducing reduced basic fuchsin (section 2.8) into the transpiration stream of the shoots. Reduced basic fuchsin was used as it is

known to stain only tissues near to those in which sap is flowing (e.g. Petty, 1970).

a) Shoots

Rhododendron shoots were allowed to develop sap tension (indicated by measurement of leaf balance pressure) by transpiration on the laboratory bench and then trimmed underwater (section 4.2.3) to remove xylem conduits containing air. The end of the stem was then transferred from water to a phial of reduced basic fuchsin, care being taken that a drop of water remained on the cut end during the transfer so that air was not admitted to the xylem. An uncavitated shoot (balance pressure = 0.83 MPa) and one cavitated shoot (balance pressure 4 MPa) were left to transpire the stain for eight hours and a second cavitated shoot (balance pressure 4 MPa) was allowed to transpire the stain overnight. Then stems were cut at intervals and the staining of the xylem examined (plates 4A, 4B and 4C respectively).

In the uncavitated stem the stain occurs in almost continuous bands, interrupted only by unstained leaf traces and rays, in the early wood of each year's growth.

In cavitated stems, whether transpiring the stain for eight hours or overnight (plates 4B and 4C), the stain occurs in patches, these patches being remnants of the bands seen in uncavitated stems. Often a coloured spot was found to be due to stain carried in a single, isolated vessel.

The fibres were unstained in all experiments.

Stain carried in the vessels did not travel as far up the stems of cavitated shoots as in the stems of uncavitated shoots.

b) Leaves

Leaves were cut underwater from shoots and set to transpire reduced basic fuchsin for 30 seconds or 5 min. The pattern of staining was the same in both cases. Sections cut from the petioles of uncavitated and cavitated leaves after infusion of the stain are shown in plate 5.

Plate 4. Staining of xylem in Rhododendron stems by reduced basic fuchsin.

A) Uncavitated shoot (balance pressure = 0.83 MPa), stained 8 hours, section cut at 1,5,10,15,20,30,50,100 mm.

B) Cavitated shoot (balance pressure > 4 MPa), stained 8 hours, sections cut at 0,5,10,15,20,25,35 mm.

C) Cavitated shoot (balance pressure > 4 MPa), stained overnight, sections cut at 0,5,10,15 mm.

(x 4.5)


A
B
C

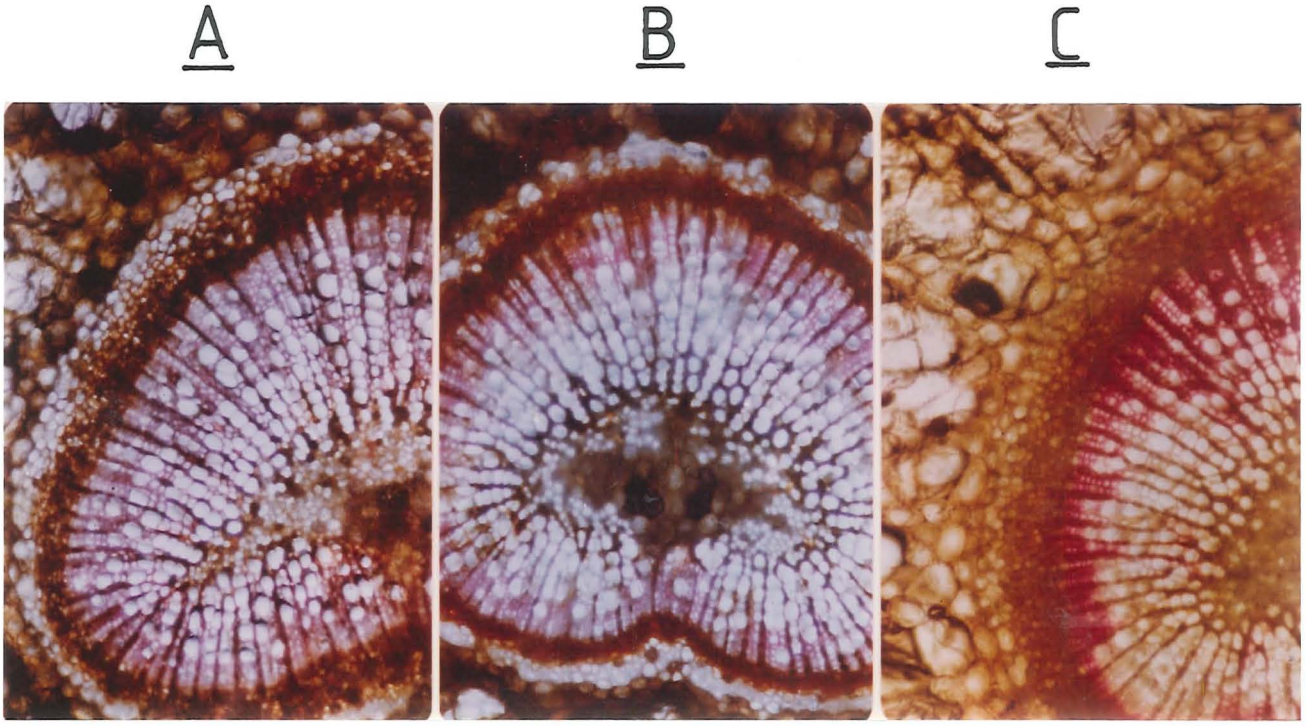


Plate 5. Staining patterns in petioles of Rhododendron leaves transpiring reduced basic fuchsin. (Approximately x 60)

A. Uncavitated leaf (balance pressure = 0-0.05 MPa).

B. Cavitated leaf (balance pressure = 2.76 MPa).

C. Cavitated and rehydrated leaf (maximum balance pressure after stress = 2.76 MPa, balance pressure after 19 hours rehydration = 0-0.05 MPa).

In uncavitated leaves the heaviest staining is found in the inner parts (mostly vessels) of the crescent shaped vascular strand (plate 5A). In cavitated leaves these vessels stained very lightly or not at all (plate 5B). The outer band of fibres, which was almost unstained in uncavitated leaves, was heavily stained in cavitated leaves. The staining patterns in leaves from cavitated shoots which had been rehydrated and recovered the ability to produce clicks were intermediate between those of the previously uncavitated or cavitated, but not rehydrated, leaves. Some of the vessels were stained as well as the fibres (plate 5C).

Attempts to stain the petiolar xylem by introducing stain into the transpiration stream of transpiring cavitated shoots were unsuccessful as dye had not reached the leaves even after several hours.

Discussion

Much less of the xylem cross-section was found to be stained in stems of shoots of high balance pressure (> 4 MPa) than in the stem of a shoot of lower balance pressure (0.83 MPa). The decrease indicates a large reduction in the number of conduits carrying sap. Similarly the staining of the vessels in leaves of low balance pressures, but not in those of high, indicates a reduction in the number of conduits carrying sap in the latter. Both observations are consistent with the hypothesis that high sap tensions may cause xylem sap to cavitate and thereby render the conduits in which cavitation has occurred unable to conduct sap.

It is thought unlikely that the differences in staining patterns of leaves and shoots were caused by air entering the cut end of the xylem or by cavitation occurring when the stems were cut as this would be expected to occur whenever the xylem sap was under tension. As all shoots and leaves had measurable sap tension (indicated by balance pressures above 0.1 MPa) air entry and artifactual cavitation would have been expected to have occurred in all these experiments but this was not the case.

The staining of the fibres of petioles of leaves of high balance pressure but not in those with low was an unexpected result and suggested that a) when vessels are unable to conduct sap some flow may occur in the fibres and b) cavitation occurs at higher sap tensions in fibres than in vessels.

Rehydration of cavitated shoots, known to restore the ability of leaves to produce clicks (section 3.6), also restored sap flow to some of the petiolar vessels. This is additional evidence for the hypothesis that 'clicks' are the result of cavitation of xylem sap.

The changes in stained areas, particularly in stems, were so large that measurable effects on the flow of sap through the stems might be expected. Experiments conducted to measure these reductions are discussed in sections 4.4, 4.5 and 4.6.

4.4. The effect of cavitation on sap flow in stem segments

4.4.1. Introduction

The staining experiments of section 4.3 have shown that in highly stressed Rhododendron shoots less of the xylem carries sap than in moderately stressed shoots. It was suggested that this reduction in conducting area was due to cavitation of the sap in the xylem, and that the decrease in the conducting area of the xylem may have measurable effects on the permeability of the stems.

In the experiments described below the effect of high sap tensions on the flow of sap in the xylem was assessed by measuring the permeability of stem segments cut from shoots over a wide range of balance pressures.

The permeability of the xylem was measured in terms of its relative conductivity (K) (Heine, 1970), defined as

Equation 2 $$K = \frac{Ql\eta}{\Delta P\, A}$$

The symbols are as defined in section 1.8.1.

The use of stem segments has advantages for the study of the effects of cavitation on sap transport.

a) The parameters required for the calculation of K, i.e. flow path length (l), xylem area (A), pressure causing flow (ΔP), and sap flux (Q), are easily measured.

b) Because the segment is relatively short it is unlikely to have differences in hydraulic conductivity along its length. However the disadvantages of using stem segments are twofold. Firstly cavitated conduits may refill as the liquid used to assess flow is at near atmospheric pressure and secondly, the xylem may become clogged by small particles or bubbles carried in the water used to make the measurements. However, as conduits may take several hours to refill (section 3.6) and as clogging can be minimised by filtration and degassing of the water used in experiments (Booker, 1977) it may be possible to determine the effects of cavitation on xylem permeability by using stem segments.

Experiments were conducted using segments of Rhododendron and Ricinus stems.

4.4.2. The electrical analogue and the flow of water in stem segments

As both the length (l) of the stem segments and the pressure (ΔP) used to drive the flow of water through them were varied in these experiments it was thought desirable to demonstrate that equation 2 did describe adequately the dependence of K on l and ΔP. It would also have been desirable to test that the relation also held for xylem area (A) but it was not possible to control xylem area in the segments used in these experiments.

The results of experiments to test the dependence of Q (and hence K) on ΔP are shown in figures 38 and 43 and on l in figure 39. Q was found to increase in direct proportion to ΔP over the range 0-4.14 MPa (Figure 38 A, C) This was similar to the results of Sucoff et al. (1965). Moreover, when extrapolated, the line passed close to the origin indicating that end effects could be neglected when calculating K (Zimmermann and Jeje, 1981). An irreversible decline in Q at high ΔP was sometimes found (figure 38B) This was due to clogging of xylem which occurred when large volumes of water were passed through

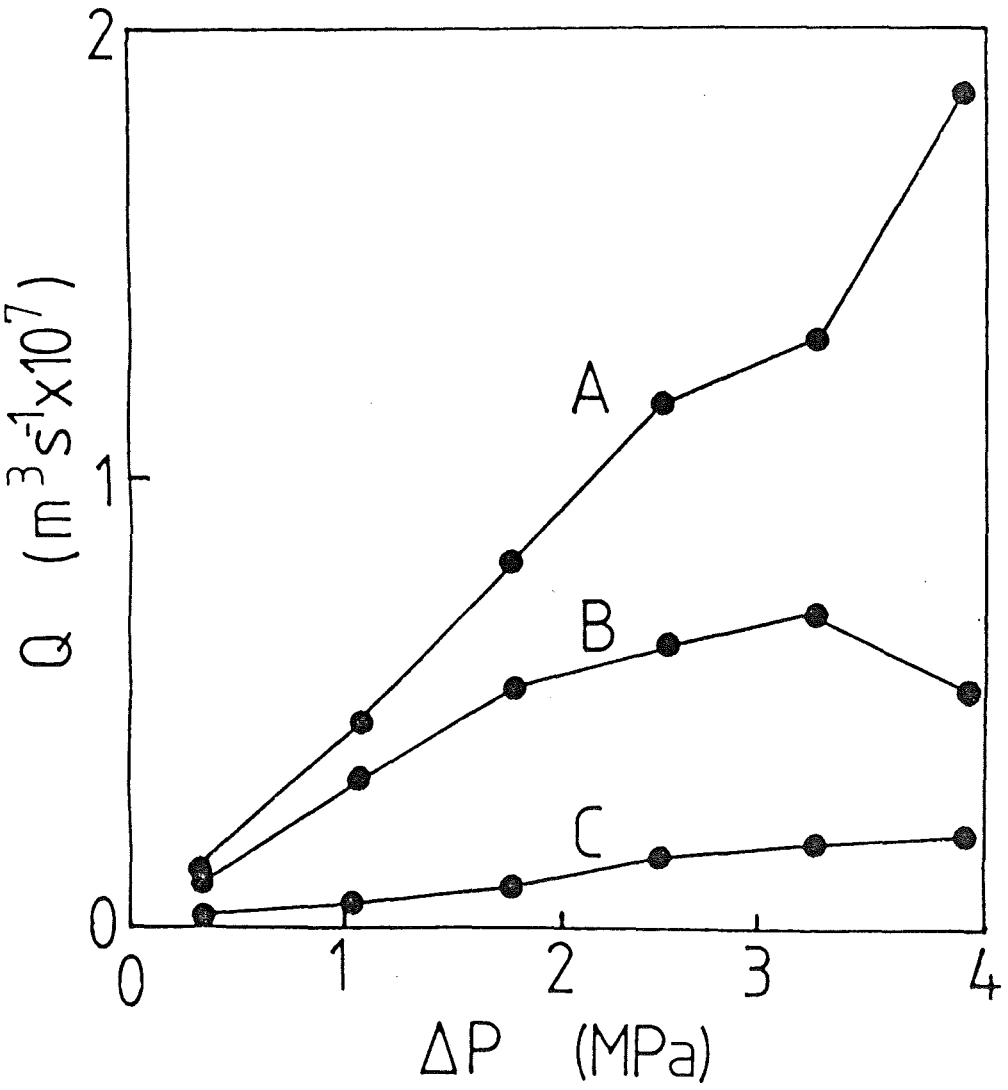


Figure 38.

The effect of changes in the pressure driving flow (ΔP) on the flow of water (Q) through Rhododendron stem segments.

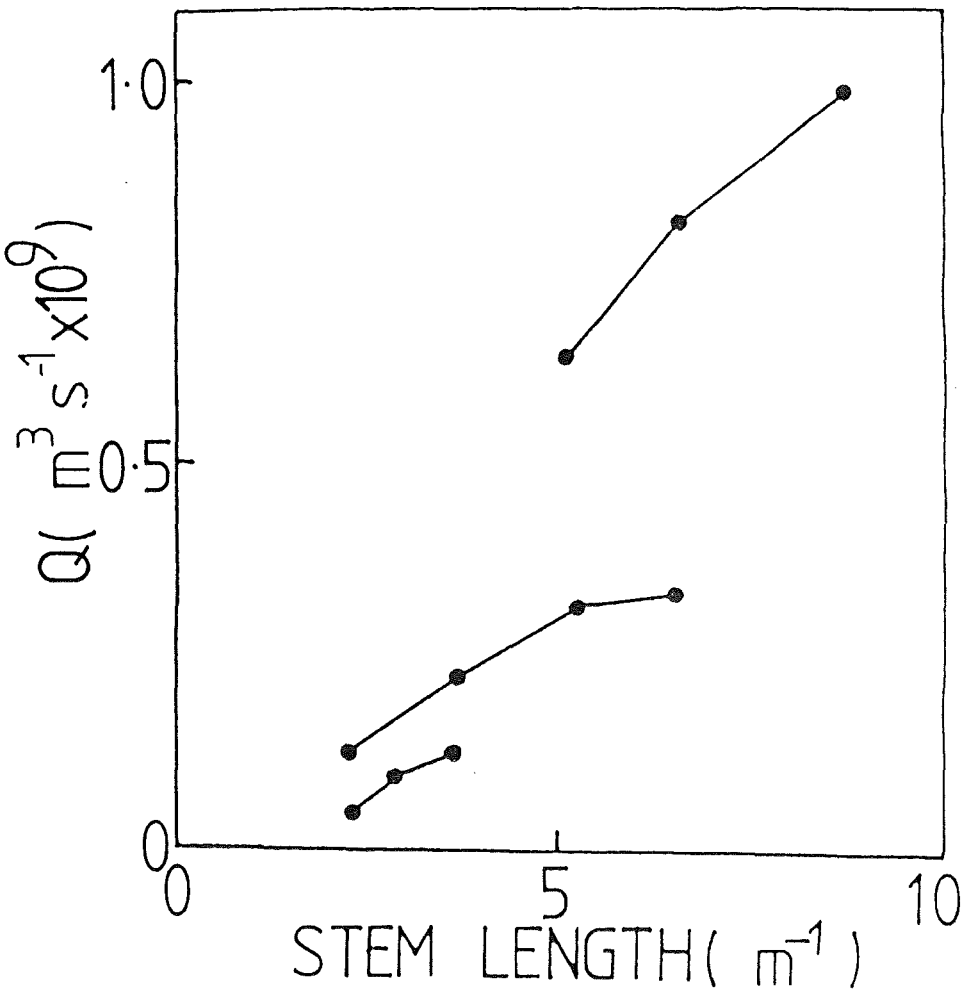


Figure 39.

The effect of reductions of stem length (1) on the flow of water (Q) through Rhododendron stem segments.

the stems. This could be greatly reduced by prefiltering the water used by passing it through 200-300 mm lengths of Rhododendron stem.

Q was found to increase slightly less rapidly than predicted when Rhododendron stems were shortened (figure 39). This was thought to result from slow, progressive clogging of the xylem occurring over the long (3-4 hours) duration of the experiments. As the observed deviations from the expected relationship were small, K values measured in stem segments 100-150 mm long could be compared directly. Stem segments shorter than 95 mm were not used in permeability experiments. Such segments may contain a significant number of conduits which extend over the length of the segment without an intervening pit membrane and which may therefore constitute a low resistance (hence high K) path for water flow.

Xylem areas could not be determined with great accuracy because of difficulty in delimiting the inner edge of the ring of xylem where it met the pith. The error resulting from this was proportionally greatest in one year old stems in which the ring of xylem was thinnest. It was estimated that errors in measurement of xylem areas were usually about 5-10% but could be higher when using very thin stems.

4.4.3. Changes in Q during experiments

There are three causes of changes in the rate of flow of water through the stem segments used in these experiments:

i) clogging of xylem conduits and pit membranes by small particles and bubbles carried in the permeating water.

ii) refilling of conduits by water under the small positive pressures used to drive flow.

iii) changes in the rate of water uptake by water stressed tissues in the stem segments.

It was found that, except for a short period of rapidly changing flow immediately after the stems were mounted in the permeability apparatus, both

the flow into (Q_{in}) and out of (Q_{out}) short stems from unstressed and stressed shoots remained nearly constant for at least an hour (figures 40 and 41). The changes in flux found in the first few minutes of experiment were believed to be mainly due to elastic relaxation of the latex tubing used to seal stems into the permeability apparatus. A cyclical variation of slightly less than $\pm$ 1 x 10^{-11} m^3 s^{-1} in measured flux rates was caused by cycling in the temperature (by $\pm$ 2K) of the room in which experiments were conducted.

Clogging and refilling of conduits was therefore not a problem when using Rhododendron stem segments to assess the effect of cavitation on xylem permeability.

It has been suggested that tyloses forming in response to cutting of stems (Murmanis, 1975) might reduce stem permeability (Edwards, 1980; Booker, 1977). As no tyloses were seen in sections cut from the stems of Rhododendron shoots kept for up to four days after sampling, tylose formation was not expected to be a factor affecting the permeability of the stems used in these experiments.

Satisfaction of water deficits in stem segments

Flow of water to satisfy the water deficits of the living cells in stem segments cut from water stressed shoots will result in elevated Q_{in} and depressed Q_{out}. Because of the large proportion of Rhododendron stem segments occupied by pith and xylem the flow to satisfy water deficits of these cells has the potential to cause errors in measurements of stem permeability.

Flow to satisfy internal water deficits of a water stressed Rhododendron stem 492mm long was shown to be sufficient to cause water to flow into both ends of the stem (figure 41). This uptake declined over several hours, as shown by a slow return to efflux from the downstream end of the stem.

Water uptake by cells in water-stressed stems was found to be a problem only when long stems were used. By stripping bark from stem segments and, bearing in mind that $Q \alpha l^{-1}$, by using short stem segments (100-150 mm long) it was possible to reduce water uptake by water-stressed tissues in the stems to

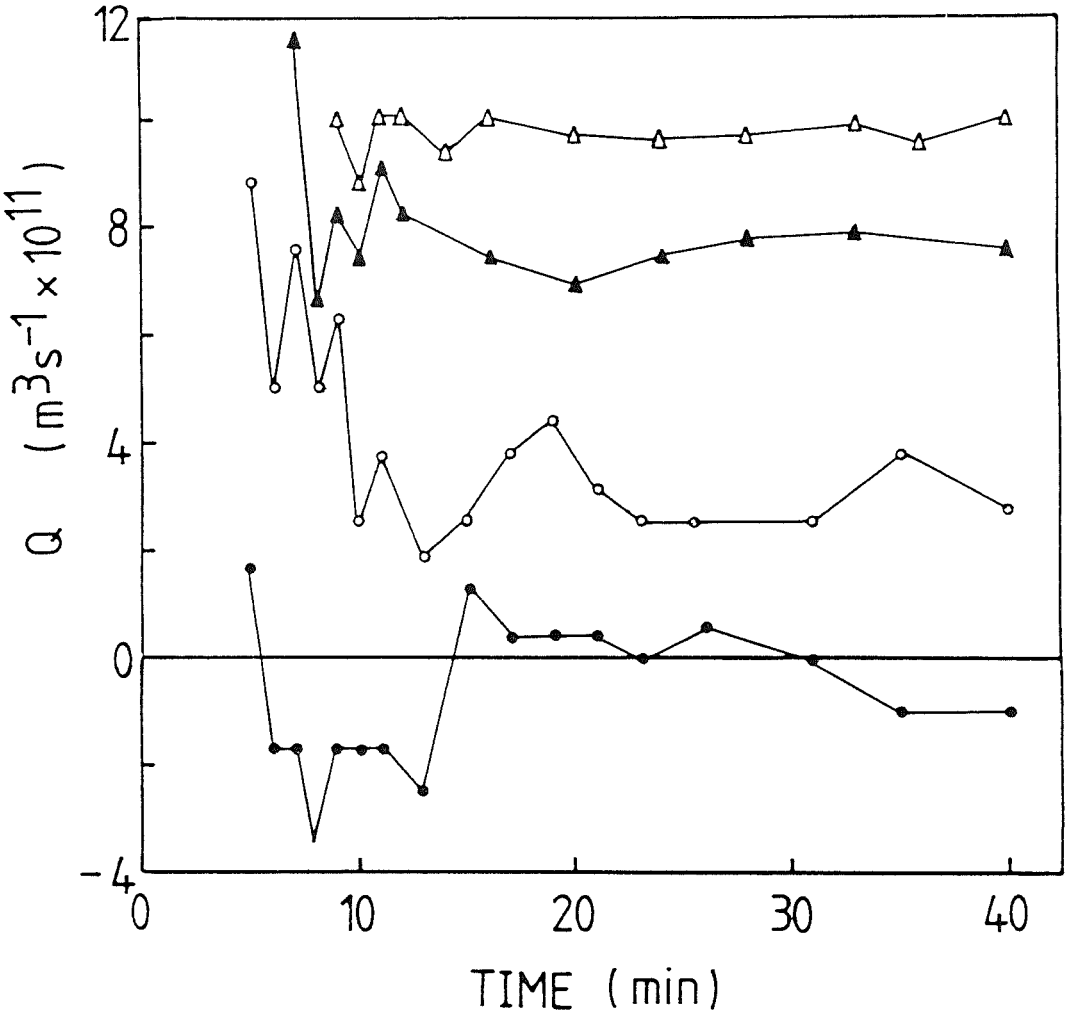


Figure 40. The flow of water into (open symbols) and out of (closed symbols) short segments of one year old Rhododendron stem from which the bark has been removed and the exposed xylem smeared with lanolin.

Δ, ▲ - From an uncavitated shoot (balance pressure = 1.22MPa, l = 113mm).

o, ● - From a cavitated shoot (" " = 2.48MPa, l = 132mm).

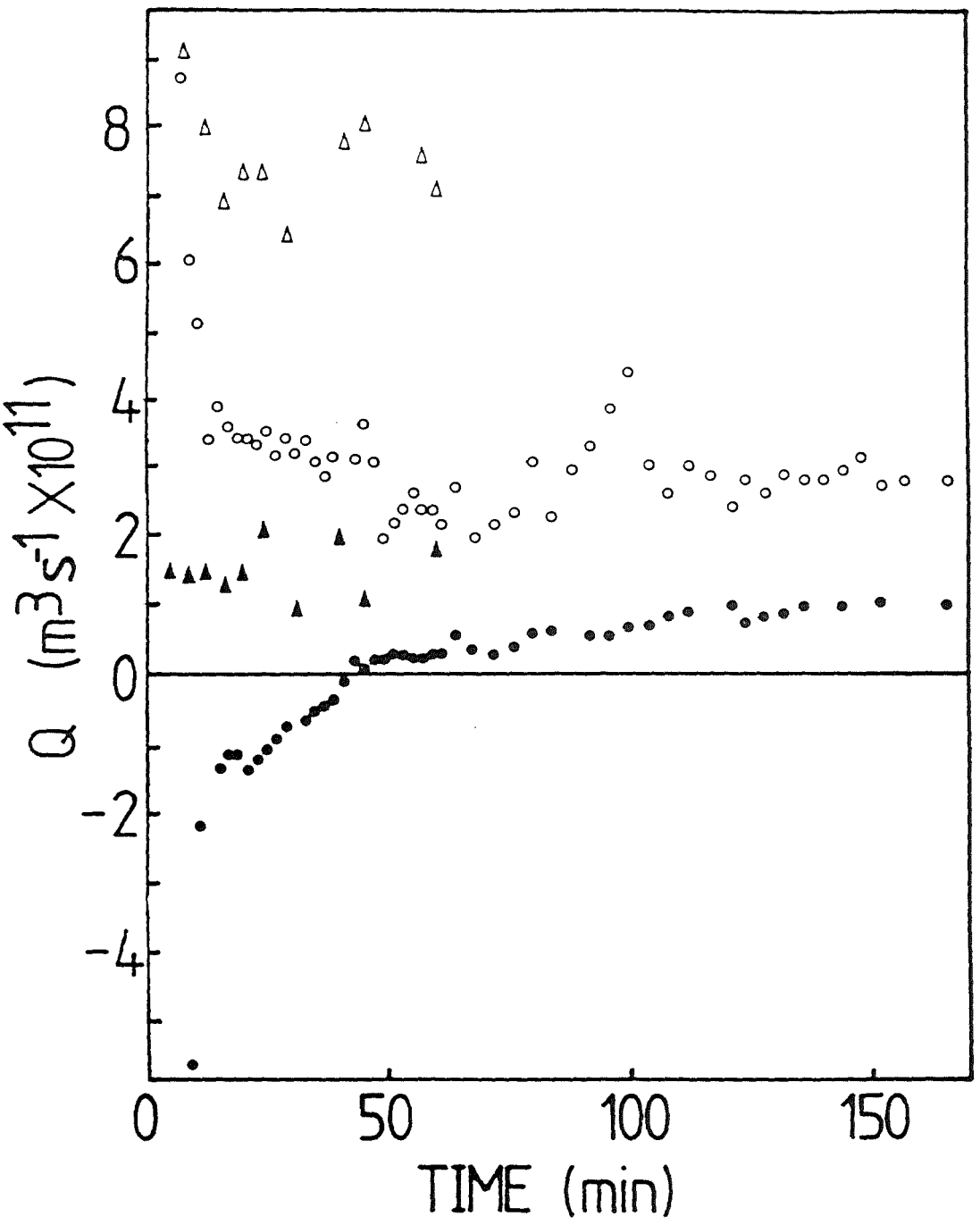


Figure 41. The flow of water into (open symbols) and out of (closed symbols) a long Rhododendron shoot.

Δ, ▲ - Turgid shoot (balance pressure = 0-0.05 MPa) 450mm long including one and two year old stems and with leaves still attached.

o, • - The same stem after stressing (balance pressure = 2.46 MPa) followed by removal of leaves and trimming of 40mm from each end of the stem.

a negligible part of the total flow through the stems (e.g. figure 40).

The remaining differences between Q_{in} and Q_{out} (e.g. figure 40) were due to leaks at the seals joining stems into the permeability apparatus and evaporation from the barkless stems even though they were smeared with lanolin. The effect of these remaining errors was minimised by averaging Q_{in} and Q_{out} to obtain Q for calculation of K. As leaks, evaporation, and uptake by water stressed tissues will tend to raise Q_{in} and decrease Q_{out} errors caused by them will be at least partially cancelled against each other by this procedure. The effect of the cycling of temperature was minimized by averaging fluxes measured over 20 to 30 minutes, excluding the first ten minutes of each experiment.

4.4.4. The effect of cavitation on relative conductivity (K)

The effect of cavitation on K was investigated in segments of stems of Rhododendron and Ricinus.

The flow of water was measured through stem segments cut from shoots which had been a) stressed to less than cavitating sap tensions, b) stressed to greater than cavitating sap tensions or c) cavitated and rehydrated.

a) Rhododendron

The majority of experiments were conducted using one and two year old shoots but a small number of three year old stems were also used. Segments were cut from the leafless parts of each season's growth to avoid complications caused by leaf traces.

The selection of a value of sap tension at which to divide stems into the cavitated and uncavitated classes was subjective. After consideration of the cavitation profiles of Rhododendron leaves (figure 28), a sap tension of 1.8 MPa (balance pressure = 1.9 MPa) was chosen. It is within ± 0.1 MPa of this tension that the greatest increase in the incidence of cavitation occurs. After stressing on the laboratory bench shoots were equilibrated in plastic bags for 30 minutes before leaves were sampled to assess sap tension (section 3.6).

Some shoots were then returned to rehydrate for 18-24 or 42-48 hours before stem segments were cut for permeability experiments.

The results of these experiments are presented in table 13. The results obtained using one and two year old shoots subjected to increasing sap tensions only are shown in figure 42.

i) Increasing sap tension

The results obtained were quite variable (figure 42). There was no sharp decline in K which would indicate extensive cavitation occurring over a narrow range of sap tensions. However a general decline in K occurred as sap tensions rose above about 1 MPa. The flow of water through the xylem had almost ceased at sap tensions of around 4 MPa. Stem segments cut from shoots which had been maintained at high levels of water deficit for a day were less permeable than those whose permeability had been measured soon after developing stress (table 13). This was associated with slightly higher sap tensions (by 0.2-0.4 MPa) in shoots kept at high water deficits for a long period and was not necessarily caused by stabilisation of cavitation emboli during the period when the stems were kept at high tension.

ii) Rehydration of stressed stems

The permeability of stem segments from shoots which had been stressed and rehydrated were similar to those of stems which had not been stressed at all. The recovery of permeability was similar whether stems had been set to rehydrate immediately upon attaining high sap tensions or a day later and was the same or only slightly greater in stems rehydrated for two days rather than one (table 13). Balance pressures of all shoots, whether rehydrated for one or for two days, were between 0 and 0.05 MPa.

iii) Flow paths in stem segments

When stained with reduced basic fuchsin no differences in the patterns of staining of the xylem of stem segments cut from non cavitated or cavitated

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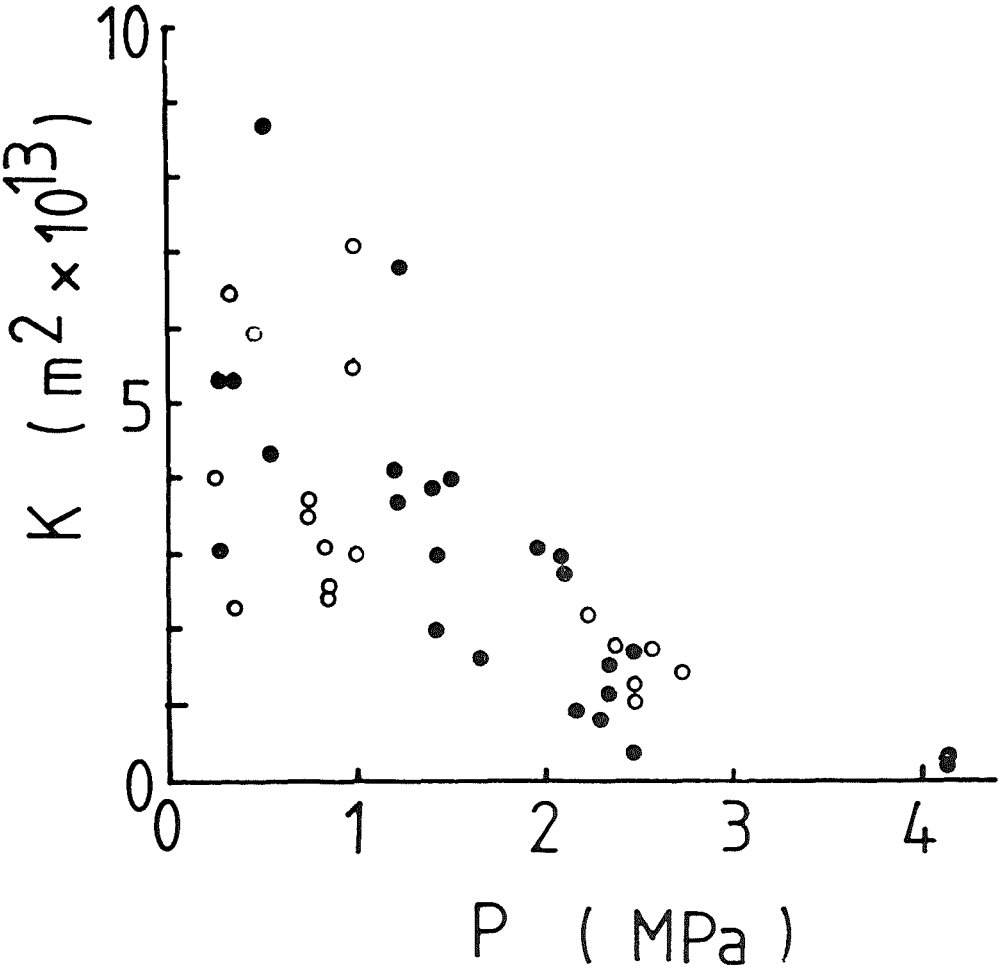


Figure 42. The effect of increasing water stress, indicated by leaf balance pressure (P), on permeability of stem segments. Permeabilities were calculated using averaged influx and efflux rates, i.e. $Q = \frac{Q_{in} + Q_{out}}{2}$

- o One year old stems.
- o Two year old stems.

TABLE 13. Permeability of stem segments cut from one, two and three year old Rhododendron shoots. Three permeability values are given for each stem class

a) K_{in} - calculated from Q_{in} (see text)

b) K_{out} - calculated from Q_{out} (see text)

c) Average K = $\frac{K_{in} + K_{out}}{Q}$

Shoots of sap tensions below 1.8 MPa were classed as 'uncavitated', those of higher sap tensions as 'cavitated'.

Values are - S.E. (number of experiments) * Values for 3 year old stems omitted

Age of shoot (years)		K ($m^2 \times 10^{13}$)						
		Uncavitated	Cavitated K measured		Cavitated and rehydrated immediately		Cavitated and rehydrated a day later	
			Immediately	Delayed overnight	1 Day	2 Days	1 Day	2 Days
1	K_{in}	5.0 ± 0.5 (13)	3.6 ± 0.9 (3)	1.8 ± 0.4 (5)	4.7 ± 1.4 (7)	6.9 ± 0.6 (2)	5.1 ± 0.2 (3)	5.2 ± 1.2 (5)
	K_{out}	3.6 ± 0.6 (11)	1.6 ± 0.8 (3)	0.8 ± 0.5 (7)	3.9 ± 1.4 (7)	6.1 ± 1.1 (2)	4.4 ± 0.3 (3)	4.7 ± 1.2 (5)
	Aver. K	4.7 ± 0.6 (11)	2.0 ± 0.9 (3)	1.3 ± 0.5 (5)	3.8 ± 1.4 (7)	6.5 ± 0.8 (2)	4.7 ± 0.2 (3)	5.0 ± 1.2 (5)
2	K_{in}	4.9 ± 0.6 (11)	2.2 ± 0.3 (3)	1.2 ± 0.4 (4)	-	-	-	-
	K_{out}	3.8 ± 1.2 (11)	1.5 ± 0.3 (3)	0.9 ± 0.3 (4)	-	-	-	-
	Aver. K	4.3 ± 0.5 (11)	1.8 ± 0.2 (3)	1.1 ± 0.3 (4)	-	-	-	-

TABLE 13 (contd.)

		Uncavitated	Immediately	Delayed overnight	1 Day	2 Days	1 Day	2 Days
3	K_{in}	4.2 ± 2.4 (3)	2.7 (1)	0.5 (1)	-	-	-	-
	K_{out}	3.9 ± 2.3 (3)	1.1 (1)	- 0.2 (1)				
	Aver. K	4.1 ± 0.9 (3)		0.1 (1)				
All measure-ments	K_{in}	4.9 ± 0.4 (27)	3.1 ± 0.8 (6)*	1.5 ± 0.3 (9)				
	K_{out}	3.7 ± 0.6 (25)	1.5 ± 0.3 (6)	0.9 ± 0.3 (9)				
	Aver. K	4.4 ± 0.4 (25)	1.8 ± 0.2 (6)	1.1 ± 0.3 (9)				
	K_{in}		1.7 ± 0.3 (15)*		6.5 ± 0.6 (9)		5.1 ± 0.2 (8)	
	K_{out}		1.2 ± 0.2 (15)		5.3 ± 0.9 (9)		4.4 ± 0.3 (8)	
	Aver. K		1.6 ± 0.2 (15)		5.7 ± 0.7 (9)		4.8 ± 0.2 (8)	
	K_{in}				6.3 ± 0.2 (17)			
	K_{out}				4.5 ± 0.3 (17)			
	Aver. K				4.8 ± 0.2 (17)			

shoots could be found in sections cut 10 and 40mm from the inlet ends. In one year old stems almost the entire xylem cross-section was stained. In two year old stems only the earlywood of each year's growth was stained.

b) Ricinus: Seven permeability experiments using Ricinus stems from plants of leaf balance pressures between 0 and 1.4 MPa were conducted. The results were very variable and no consistent effects of high sap tensions on the permeability of these stems could be found. Because of this, and because flow of water to the large pith and its central pores may have been affecting results, no further experiments with Ricinus were attempted.

4.4.5. Introduction of emboli into xylem by injection of gas

In the experiments described above the filling of xylem conduits by emboli formed by cavitation has been suggested as the cause of decreases in stem permeability and of the conducting area of the xylem. The experiments in this section were conducted to demonstrate that embolisms introduced into the xylem artificially had effects on sap conduction similar to those of high sap tensions.

The pressure chamber was used a) to fill xylem conduits with nitrogen gas and b) to refill the conduits with water (by the procedure of section 2.5.1).

A 100mm segment of Rhododendron stem was cut from a shoot of balance pressure 2.29 MPa, mounted in the permeability apparatus and the flow of water through it measured after each of the following sequence of treatments:

a) 6 hours submerged under distilled water at atmospheric pressure.

b) applying nitrogen gas at 2.5 MPa pressure to one end for 6 minutes.

c) passing filtered water through the stem at 0.5 MPa pressure for 20 minutes, 40 minutes or 60 minutes.

Typical results are shown in figure 43.

From balance pressure measurements it was known that the stem was partially cavitated at the beginning of the experiment. After six hours of rehydration stem permeability had increased by 30%, indicating some refilling of conduits

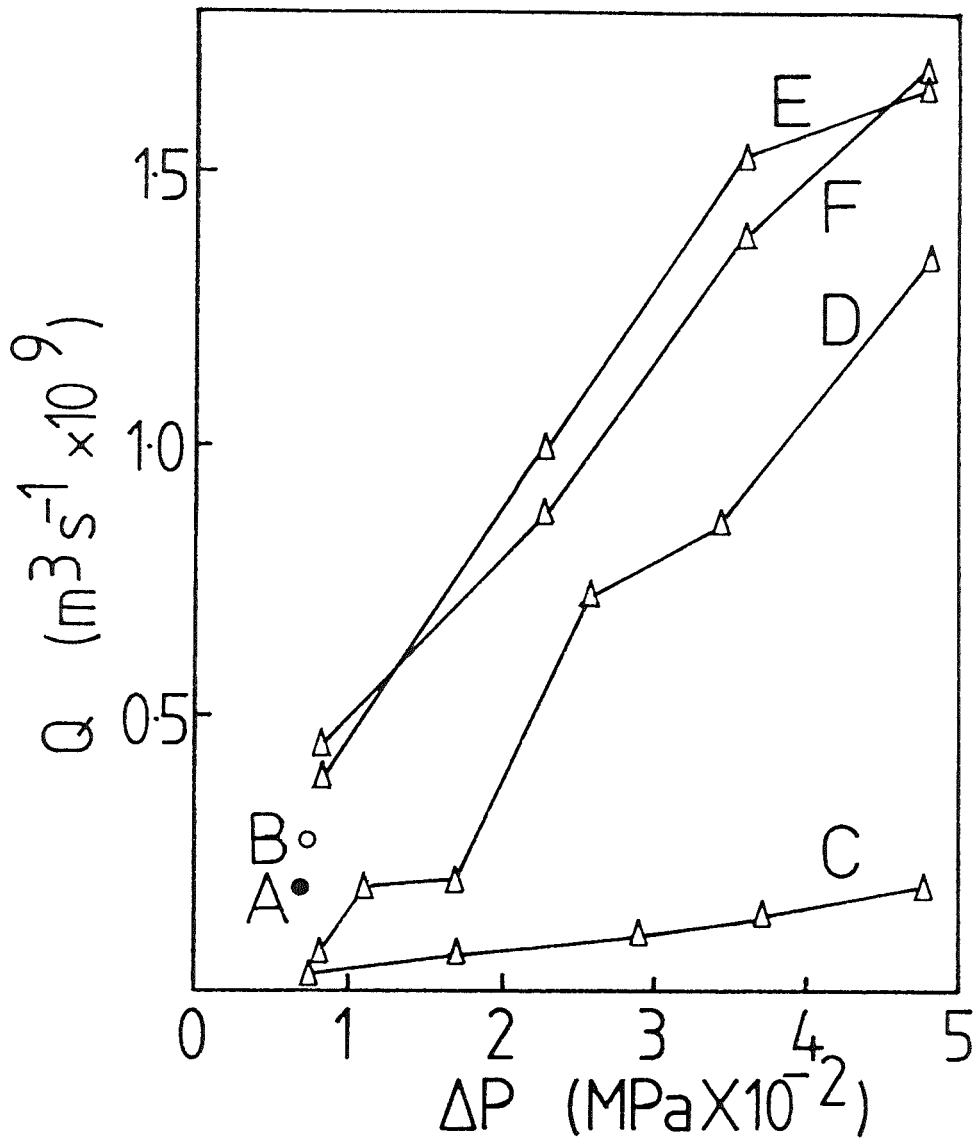


Figure 43. The effect of injection of gas or water on the flow of water through a segment of Rhododendron stem.

A - Immediately after cutting from water stressed plant (balance pressure = 2.29 MPa).

B - After 6 hours soaking in distilled water.

C - After injection of nitrogen gas at 2.5 MPa for 5 minutes.

D - " " " water at 0.5 MPa for 20 minutes.

E - " " " " " 0.5 MPa " 40 minutes.

F - " " " " " 0.5 MPa " 60 minutes.

The pressure driving the flow of water (ΔP) was varied between 0.007 and 0.05 MPa by applying a vacuum to the outlet end of the stem segment.

in the segment. Injection of nitrogen reduced flow of water through the stem almost to zero. Forced refilling of xylem conduits by injection of water under pressure increased the ability of the stem to pass water. In the three experiments of this series the flow of water through the stems was increased to 2 to 2.5 times that when flow through the segment was first measured. This is a similar degree of recovery of permeability to that found when cavitated Rhododendron shoots were rehydrated (table 13). In two of the experiments, including that of figure 43, an apparent upper limit to the permeability of the stem segments was reached after several injections of water under pressure. This may be because all conduits in the stem have become refilled and are now carrying water or because clogging of the xylem was preventing further increases in sap flow.

4.4.6. Discussion

Uncavitated Rhododendron stems were found by the above experiments to have relative conductivities of about 5×10^{-13} m^2 (table 13). These were only slightly lower than the 6×10^{-13} m^2 determined for Rhododendron ponticum by Farmer (1918).

The permeability of Rhododendron stems decreased as sap tensions increased but returned to near that of unstressed stems when rehydrated for a day or more (table 13). Stem permeability decreased over the entire range of sap tensions between 0 and 4 MPa but the most marked decreases occurred at sap tensions between 1 and 3 MPa. Similar, although larger and more rapid, changes in permeability can be induced by injecting gas or water under pressure into stems (figure 43). These results are in broad agreement with those of acoustic experiments (sections 3.5 and 3.6) and are consistent with the hypothesis that cavitation occurring at sap tensions between 1 and 2.5 MPa renders xylem conduits unable to conduct sap.

However it is not possible to discount the alternative hypothesis that cavitation or air entry occurring during preparation of the samples for permeability measurements was responsible for decreases in permeability. The

broad range of sap tensions over which the decreases in permeability occur may therefore only indicate that artifacts (and especially cavitation caused by cutting of stems) are more likely to occur at high rather than at low sap tensions. This possibility may be tested by subjecting sap in the xylem to known sap tensions and then by rehydrating shoots so that sap tensions fall but, by not allowing sap pressure to become positive, preventing cavitation emboli dissolving (section 3.6), and cutting stem segments from both cavitated and uncavitated shoots or plants at a time when sap tensions are uniformly low. This approach was attempted in experiments with whole plants (section 4.6) and indicates that the observed decreases in permeability were not due to cavitation occurring during preparation.

The similarity of staining patterns in stem segments from shoots of high and low sap tensions (section 4.4.5) is in contrast to the results of the staining experiments using shoots (section 4.3). There are two possible explanations but, in the absence of further evidence, no choice can be made between them.

Firstly, both acoustic and permeability experiments show cavitation to occur progressively as sap tensions increase. Therefore, at sap tensions between 1 and 3 MPa, a sufficient number of conduits may remain uncavitated for the stained areas around them to overlap and to give the appearance that the stain was carried in all conduits. However the shoots used in section 4.3 had sap tensions of over 4 MPa and cavitation would be almost complete at these sap tensions (table 9). The stained areas around the remaining sap-filled conduits would not overlap and much of the xylem will remain unstained with only spots of colour around the few remaining uncavitated conduits.

Alternatively, because permeability experiments were conducted using water at greater than atmospheric pressure, emboli may have been compressed or partly dissolved during the experiments and so admitted stain to even cavitated conduits. However even a small bubble will prevent flow through the large pit fields at the end of the conduits. Therefore flow from the conduit must be through the high resistances of the bordered pits on the walls of the conduits,

resulting in the lower permeabilities of stems from shoots of high sap tensions.

4.5. Cavitation and the uptake of water by shoots

4.5.1. Introduction

The experiments described in section 4.4 show stem permeability to decrease as water deficits increase. However there were indications, for instance in staining patterns, that conduits were at least partially refilled during these experiments. Refilling of conduits occurring during permeability measurements can be avoided by reducing the pressure of the xylem sap to less than that of the bubbles in the xylem conduits (section 3.6).

A reduction in sap pressure occurs whenever moving sap passes a resistance to flow (Richter, 1973). Form drag at pit membranes and friction at cell walls are two such resistances (Tyree and Zimmermann, 1971). Measurements of conduit lengths have shown that most of the water taken up by Rhododendron shoots will have to pass through a pit membrane within 40mm of entering through the cut end of the shoot (section 4.2). In the course of passing these pit membranes pressure within the sap will be reduced and cavitation emboli in the remainder of the xylem will be prevented from dissolving for as long as a sufficient gradient of pressure, caused by continued uptake of water, is maintained.

Because the gradient of water potential which drives water uptake starts to fall as soon as any water is taken up by the shoot a method of determining the rate of water uptake by the shoots at a time of known, uniform shoot water potential was required. This was achieved by using an electrical analogue, based on the charging of a capacitor through a resistor, to describe water uptake by a shoot (Landsberg et al., 1976). From this model the rate of water uptake by the shoot at the time water was first supplied to the shoot (i.e. when the water stressed shoot was first trimmed under water, t = 0) was calculated. The initial rate of water uptake (Q) was then combined with

measurements of shoot length, l, (section 2.5.2) and shoot water potential, ΔP, (obtained from leaf balance pressure measurements (section 3.6)) to obtain K', a measure of stem permeability (defined by equation 6) and equivalent to the relative conductivity, K, multiplied by the area of xylem in the stem of the shoot.

Eqn. 6. $$K' = \frac{Ql\eta}{\Delta P}$$

Q at t = 0 depends only on the permeability of the shoot and the gradient of water potentials between the potometer and the cells of the shoot. Therefore Q, and K' determined from it, are independent of the size of the shoot, allowing K' values to be used to compare the permeabilities of shoots from which leaves or portions of stems have been cut.

4.5.2. The effect of cavitation on the uptake of water by Rhododendron shoots

Potometers were used to measure water uptake by uncavitated, cavitated and rehydrated shoots as described in section 2.5.2.

By using long shoots it was sometimes possible to measure water uptake several times on a single shoot which had been subjected to successive treatments.

Water uptake by one such shoot is shown in figure 44. Water uptake increased as sap tensions rose from 1.46 to 1.92 MPa but decreased when a further increase from 1.92 to 2.43 MPa was imposed. Reduced rates of water uptake by shoots subjected to high sap tension were found in all five experiments of this type.

That the rates of water uptake by shoots were reduced even though the gradient of water potential driving the uptake has increased suggests that the resistance to the flow of water in the stem has increased. By assuming that the change in resistance occurs in the xylem the effect of high water deficits on water uptake by shoots was quantified by calculating K' for each experiment.

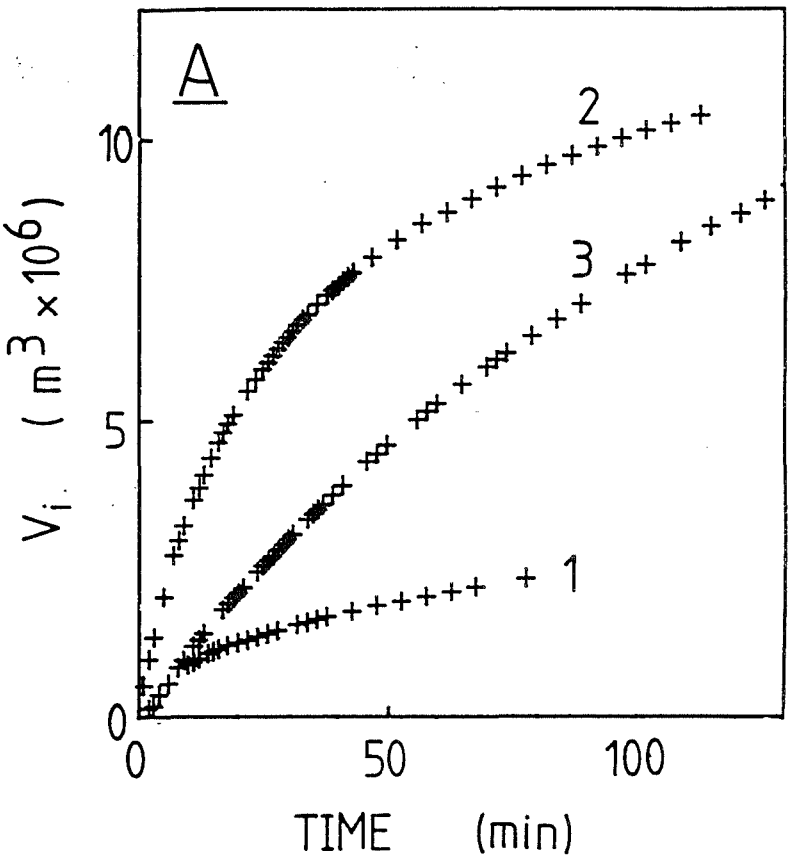
Because of the likely complexity of water exchange within the shoot during

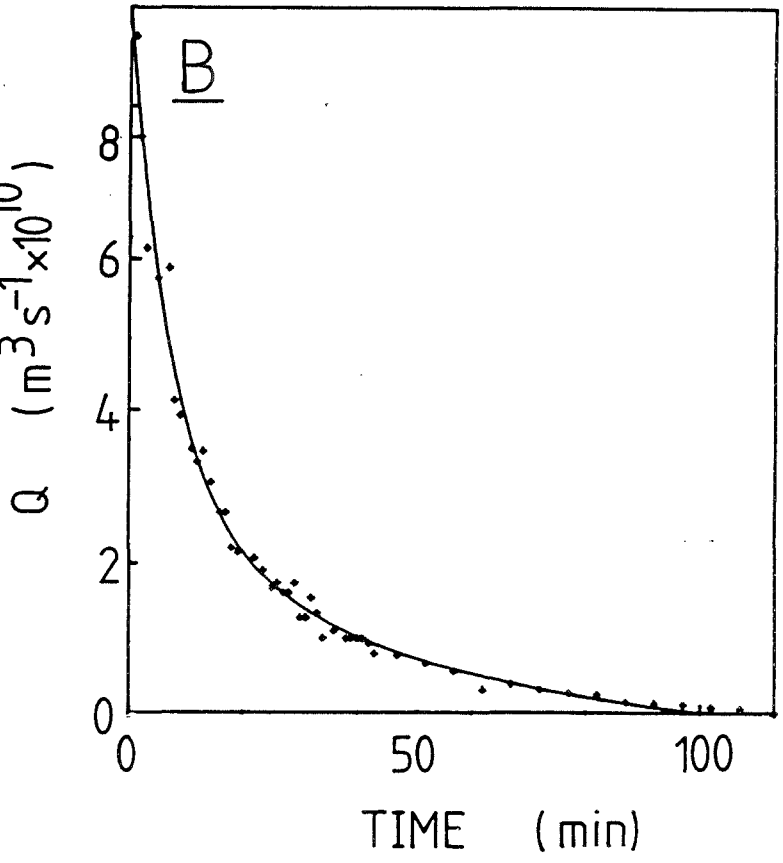
Figure 44. The effect of water stress on the uptake of water by Rhododendron shoots.

A. Cumulative volumes of water (V) taken up by the same shoot after stressing to successively higher levels.

1) Leaf balance pressure = 0.46 MPa.
2) Leaf balance pressure = 1.92 MPa.
3) Leaf balance pressure = 2.43 MPa.

B. The fit of the function described by the two capacitor model of water uptake by shoots to the data of experiment two of part A.


A
2
3
1
10
5
0
0
50
100
TIME (min)
V_i ($m^3 \times 10^6$)


B
8
6
4
2
0
0
50
100
TIME (min)
Q ($m^3 s^{-1} \times 10^{10}$)

rehydration (Tyree and Dainty, 1973), water uptake by the shoot was described using as a model two capacitors, each charging through a separate resistor (equation 11).

Eqn. 11. $$Q = (a_1.e^{-b_1 t} + a_2.e^{-b_2 t})$$

A computer-assisted least-squares procedure was used to find the best fit of this function to the data of each experiment and to calculate Q at t = 0.

The results of these analyses are shown in table 14 and figure 45. In most cases 95% confidence intervals in the rate of water uptake at t = 0, i.e. Q in equation 11, were ± 20% or less of the value of Q (e.g. table 14).

Increasing sap tension to between 1.5 and 3 MPa resulted in decreases in K' of at least 60% and usually of 80-90%. Overnight rehydration restored K' only slightly (figure 45). In several instances shoots which had been rehydrated overnight were used in two potometer experiments only hours apart. K' values were similar in both measurements, an indication that shortening the stem did not greatly affect the K' value calculated for each shoot. The large changes in K' found in shoots as water stress increased were therefore probably caused by something other than the trimming of the shoot between experiments.

4.5.3. Artifacts in potometer experiments

i) Faults in technique

Leaks from the potometer were prevented by tying a loop of string around the latex rubber tube where it overlay the stem. Evaporative losses from shoots on the potometers were about 3×10^{-12} m^3 s^{-1} (0.2 µl min^{-1}) and were therefore negligible in comparison to the uptake of water by the shoot.

Cavitation may have occurred when the water stressed shoots were being trimmed prior to connection to the potometers (Crafts, 1939). Such cavitation, if it had occurred, was not thought to have had a significant effect on the results of these experiments as such recently formed emboli would contain only water vapour at low pressure (Huber, 1956) and would collapse almost immediately when water at atmospheric pressure was made available to the xylem. That the

Table 14. The calculation of the permeability (K') of Rhododendron shoots from the electrical analogue.

The function used to determine the rate of uptake of water at the beginning of potometer experiments was (equation 11) $Q = (a_1.e^{-b_1 t} + a_2.e^{-b_2 t})$. Shown are the results and the derived parameters from which K' was calculated for two shoots. K' values for other experiments are summarised in figure 45. (See section 2.5.2 for derivation of K')

Shoot number	Maximum sap tension (MPa)	Rehydration period, if any (hours)	K' ± 95% confidence interval ($m^4 x10^{19}$)		ΔP (MPa)	a_1 ($m^3 s^{-1} x10^{10}$)	b_1 ($s^{-1} x10^3$)	a_2 ($m^3 s^{-1} x10^{10}$)	b_2 ($s^{-1} x10^4$)
8 a	0.46	0	1.34±.14	200	0.46	2.8 ± 0.3	2.1 ± .5	0.3 ± 0.2	0.3 ± 2.4
b	1.92	0	0.77±.04	160	1.92	6.2 ± 0.8	2.2 ± .5	3.0 ± 0.8	3.4 ± 1.0
c	2.43	0	0.22±.03	120	2.43	2.8 ± 0.5	3.0 ± .7	1.6 ± 0.1	3.4 ± 1.0
20 a	2.39	0	0.37±.11	145	2.39	3.0 ± 1.8	3.8 ± 3.1	3.0 ± 0.6	2.7 ± 0.9
b	2.39	20	0.92±.12	105	0.40	3.0 ± 0.4	5.0 ± 0.8	0.7 ± 0.1	5.2 ± 0.9
c	2.39	20	1.03±.29	65	0.39	5.5 ± 1.7	10.1 ± 1.6	0.6 ± 0.1	5.8 ± 0.7

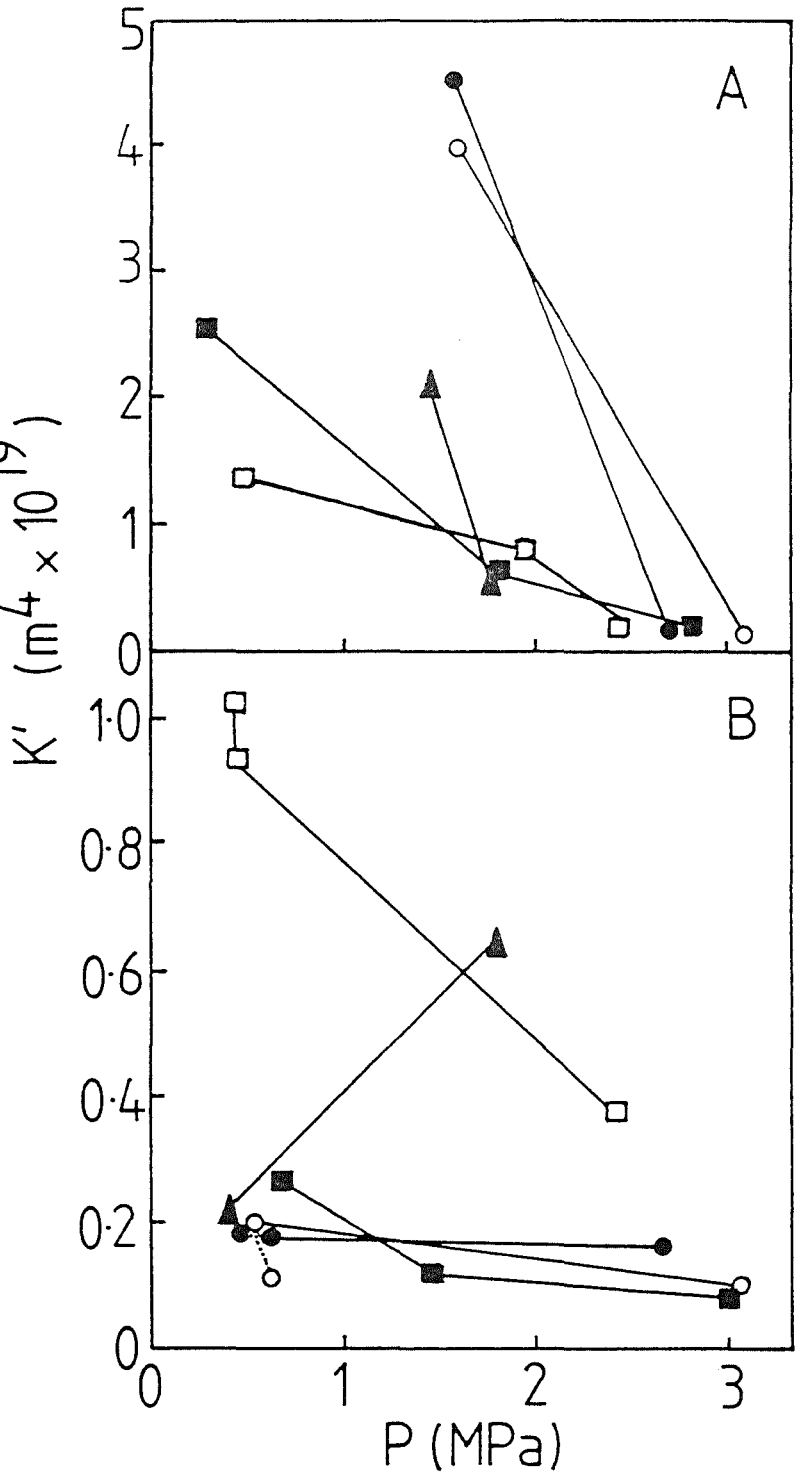


Figure 45. The effect of increasing water stress, indicated by leaf balance pressure (P), on permeability of Rhododendron shoots.

A - Increasing water stress.

B - Decreasing water stress (rehydration).

Solid lines join measurements made before and after overnight rehydration.

Dotted lines join measurements made approximately three hours apart (see text).

Open symbols refer to successive measurements using the same shoot in both A and B. Closed symbols refer to different shoots in A and B.

permeability of shoots which had been subjected to high sap tensions but then rehydrated was as low as in shoots at high sap tension supports the view that cavitation during sample preparation can be disregarded as a source of error in these experiments.

ii) The electrical analogue and water exchange by small samples

Of more concern were the inadequacies of the capacitor-resistor model used in determining the initial rate of water uptake by shoots.

There is no reason to suspect that the kinetics of water exchange depends on whether water is flowing into or out of the cells of the leaf or shoot. A study of the kinetics of sap expression by a leaf or shoot subjected to over-pressure in the pressure chamber may therefore be used to test the applicability of the capacitor analogy to the uptake of water by similar samples. Leaves were used in the following experiments because evaporation from them could be limited more effectively than could evaporation from shoots. However, as leaves are smaller and simpler in structure than shoots, a simpler model might describe water exchange by leaves than shoots (Tyree and Dainty, 1973).

Turgid Rhododendron leaves were mounted in the pressure chamber and subjected to three pressure increments. After each increment sap expressed from the petiole was collected over timed intervals and the rate of sap expression, $\frac{\Delta Ve}{\Delta t}$, determined. The cumulative volumes of sap collected in one such experiment are shown in figure 46. A least-squares fit of the function described by equation 7, i.e. $E = a.e^{-bt}$, to the expression data (obtained after each pressure increment) was found and the values for the co-efficients a and b and the half time of the expression ($= \frac{0.693}{b}$ calculated (table 15)).

The high regression co-efficients for the experiments indicate that water exchange between the xylem and cells of leaves in response to a suddenly established gradient of Ψ is closely analogous to a single capacitor charging through a resistor. However leaves are relatively small, homogenous structures when compared to shoots. Therefore a model using two (as in the experiments described above) or three capacitor resistance pairs may be more appropriate to

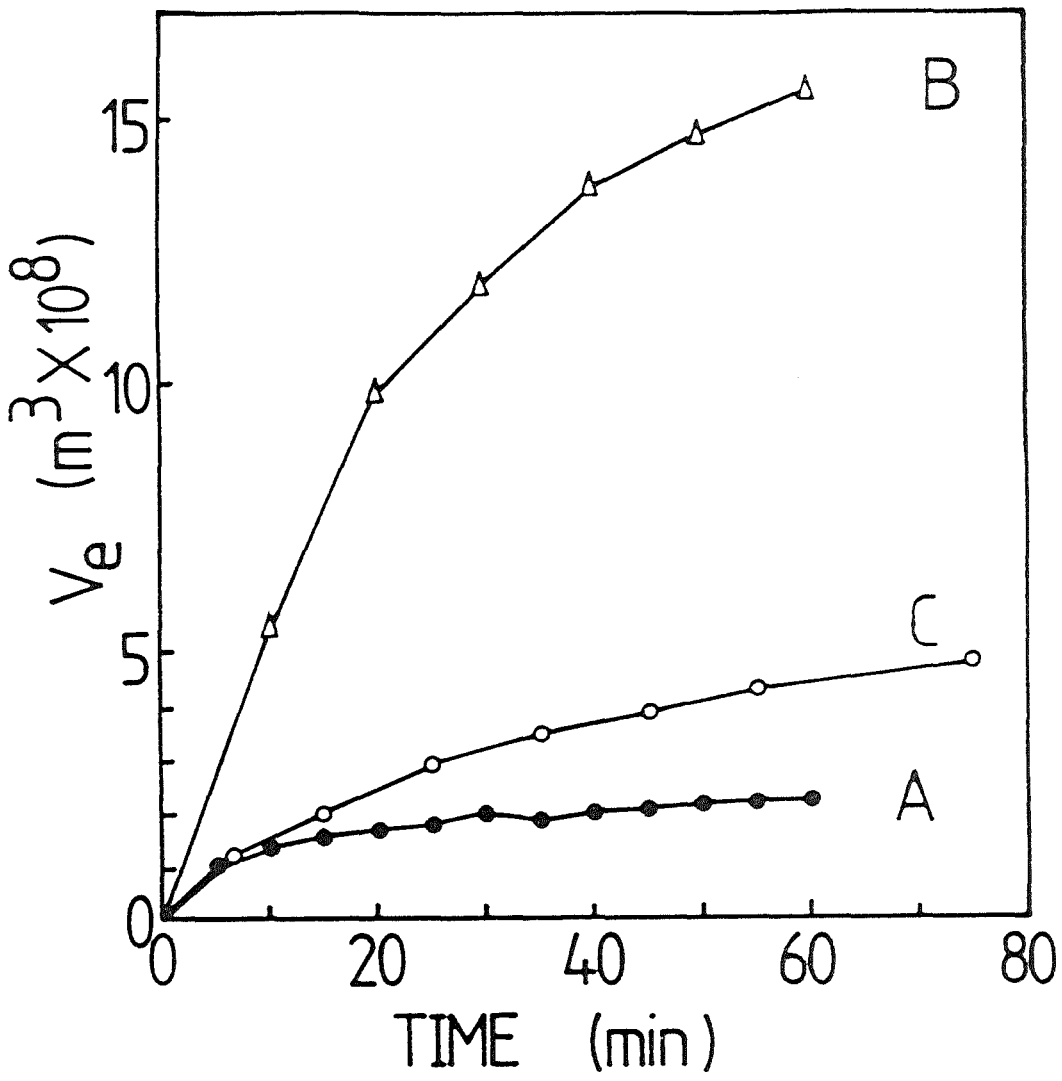


Figure 46. Cumulative volumes of sap expressed from a single, initially turgid, Rhododendron leaf after the following pressure increment:

A 0.03 - 0.21 MPa.

B 0.21 - 1.93 MPa.

C 1.88 - 2.06 MPa.

The lower pressure in each increment was the balance pressure of the leaf measured immediately before the pressure increment was applied.

discussions of water exchange by shoots even though each cell population in the shoot may be adequately modelled by a single capacitor/resistor pair (Tyree and Dainty, 1973; Roberts, 1979).

iii) Complexity of water exchange within the shoot

It was assumed when applying the analogy of several capacitor-resistor pairs to water uptake by a shoot that the water potentials of all cells of the cell populations represented by each capacitor-resistor pair changed in unison. In fact, as shown by comparison of the balance pressures of adjacent leaves (table 15) steep gradients of water potential (up to 7 MPa m^{-1}) could exist along rehydrating shoots. Moreover, local restrictions within the shoot can further complicate the rehydration of the shoot. For example the slow rehydration of leaf 1 on shoot 13 (see table 16) was the result of its petiole being bent when the shoot was mounted on the potometer.

Table 15. The kinetics of sap expression by a Rhododendron leaf subjected to three successive increments in chamber pressure. The function $E = a.e^{-bt}$ was fitted to the rate of sap expression by the leaf after each pressure increment. The parameters a, b, and the half-time of the expression, $t_{\frac{1}{2}}$, of the fitted function are shown. Also given are the total volume of sap expressed by each expression, Ve, and the evaporative loss from the leaf, as % Ve.

Expression	ΔP (MPa)	A ($kg\ s^{-1} \times 10^{8}$)	B ($s \times 10^{4}$)	$t_{\frac{1}{2}}$ (s)	r^2 of least square fit of function to data	Ve (g)	Evaporation (% Ve)
a	0.34-0.21	1.4	7.5	922	59	.022	
b	0.21-1.86	14.3	6.7	1022	96	.156	
c	1.79-1.97	.2	2.9	2432	92	.053	
					Total Ve =	.232	5.6

Table 16. Balance pressures of adjacent leaves about 10mm apart cut from within the terminal cluster of leaves of Rhododendron shoots rehydrating on potometers from the original balance pressures and for the times indicated.

Shoot number	Initial leaf balance pressure (MPa)	Duration of rehydration (min)	Balance pressures of leaves from within terminal cluster (numbered from basal end) (MPa)		
			1	2	3
14	2.18	15	0.79	1.03	1.36
13	2.17	52	0.88	0.55	0.86
12	2.84	64	1.55	2.28	

The electrical analogy used in the calculation of K', i.e. of a resistor (the stem) passing a current (the flux of water) also assumed that the sinks to which water was flowing were in a small group contacting the xylem at a single point at a distance from the supply of water. However, as shown in the following experiments, the sinks to which water flows are distributed along the stem as well as being in the leaves and probably include the bark, pith and xylem parenchyma.

Four Rhododendron shoots were stressed until leaf balance pressures were between 1.7 and 3 MPa and then connected to the potometers in the usual way. After 30-40 minutes all the leaves were removed from the shoots, causing an immediate mean reduction of 46% (S.E. = ± 5%) in the rate of water uptake by the shoots (figure 47). In two of the experiments the bark was subsequently removed as well, reducing water uptake by 25% and 33% respectively below that prior to removal of the bark.

Finally, if shoots have been water stressed for a long period, water may also be taken up to satisfy 'growth deficits' which may occur in the stem (Milburn and Weatherly, 1971).

The complexity of water exchange within shoots was probably the major restriction on the accuracy of the potometric technique for assessing the

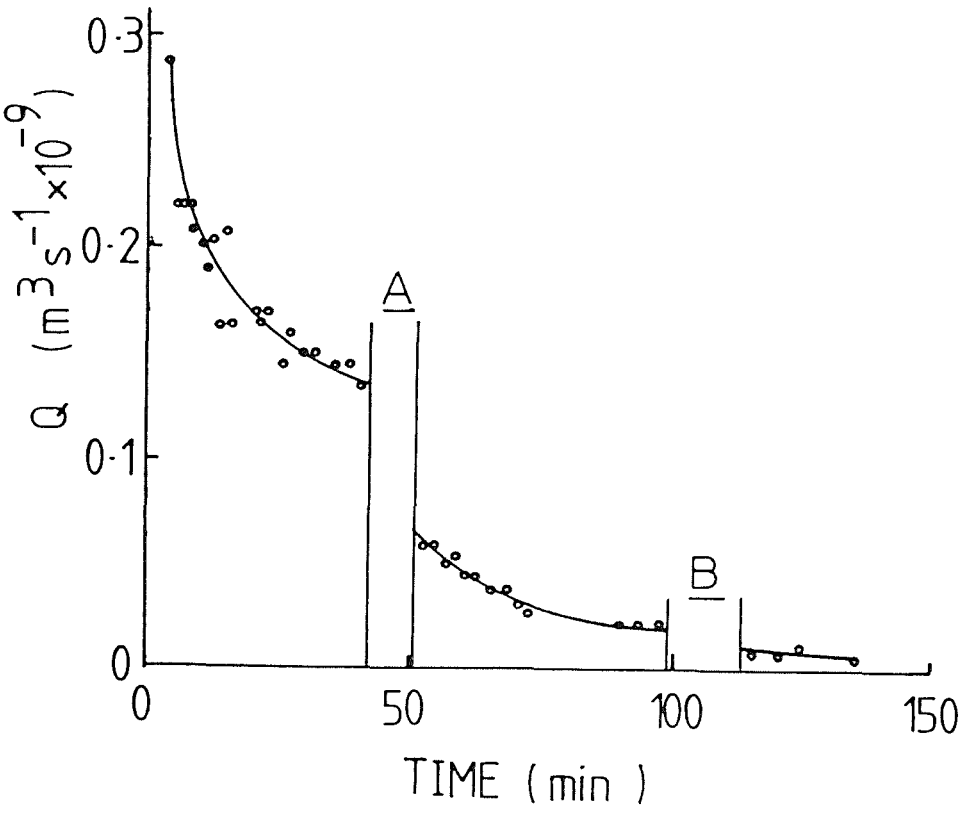


Figure 47. Reductions in the rate of water uptake by a Rhododendron shoot (initial balance pressure = 2.17 MPa) when:

A - All leaves removed.

B - Bark removed.

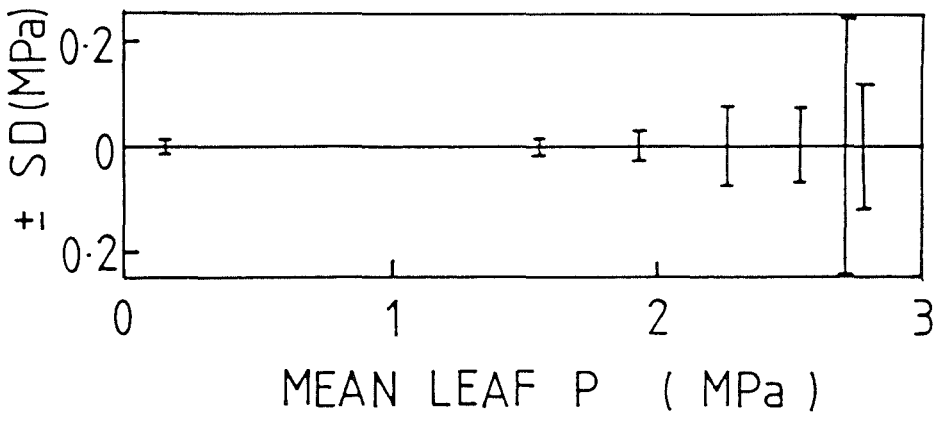


Figure 48. The difference between balance pressures of leaves sampled at the same time from shoots which had been equilibrated in plastic bags for 30 minutes after stressing on the laboratory bench.

effects of cavitation on xylem permeability.

However the differences in permeabilities between treatments were thought to be sufficiently large that the limitations of the model of water uptake used was not likely to affect the validity of the conclusions drawn from its use.

iv) Problems in determining ΔP

Two small problems were found when determining sap tension in shoots.

a) Balance pressures of leaves attached to highly stressed shoots failed to equalize during the half hour equilibration period before sampling (see section 3.6.2), making measurements of sap tension less precise as water deficits increased. Differences in balance pressures between leaves were small up to balance pressure of 2 MPa but increased rapidly as water deficits rose beyond this (figure 48).

b) A second problem was found when attempting to obtain balance pressures of leaves from rehydrated shoots. Balance pressures of some leaves from these shoots fell over several minutes until a constant but much lower balance pressure was obtained (table 17).

Not all leaves, even amongst those from the same rehydrated shoot, showed this fall in balance pressure and no similar problems were encountered when using leaves from water-stressed shoots which had not been rehydrated. The cause of this fall in balance pressures was not known. Leaves showing this behaviour were omitted from calculations of the mean balance pressure (ΔP) used in calculations of K'.

Table 17. Apparent successive balance pressures (measured 30-60 seconds apart) of leaves from stressed and rehydrated Rhododendron shoots.

Shoot number	Balance pressure before rehydration (MPa)	Mean leaf balance pressure after rehydration (MPa)	Successive balance pressure of one leaf from rehydrated shoot (MPa)						
			Measurement number						
			1	2	3	4	5	6	7
50[1]	2.86	0.59	1.00	0.90	0.76	0.62	0.28	0.55	0.55
55(1)[1]	2.50	0.74	0.67	0.62	0.52	0.32	0.35		
55(2)[2]	2.50	0.23	1.03	0.69	0.41	0.35	0.14		

1 Shoot rehydrated for 20 hours; 2 Shoot rehydrated for 48 hours

4.5.4. Discussion

The rate at which Rhododendron shoots took up water was found to decline as sap tensions rose above 1.5 MPa. This decline occurred even though the water potential gradients driving flow had increased. In quantifying the reduction in the rate uptake of water it was assumed that the reduction in the rate was due to changes in resistances located entirely within the stem. In this way it was shown that the permeability of the stem had decreased by at least 60% and usually by 80% or more as sap tension rose towards 3 MPa from around 1.5 MPa or less.

These decreases in xylem permeability are in accordance with the hypothesis that cavitation occurring at sap tensions above about 1.5 MPa may be extensive enough to restrict the flow of water in the xylem.

This agrees with the results of acoustic (section 3.5) and staining experiments (section 4.3) which indicate that cavitation can occur at similar sap tensions and with measurements of the permeability with stem segments (section 4.4). It also agrees with the increasing differences between leaf balance pressures (section 4.5.3) which show major decreases in xylem permeability at similar sap tensions.

It is interesting to compare the permeability of Rhododendron shoots with those of stem segments, not only to show the effect of high sap tensions on each but also as an aid to speculation as to the contribution of the xylem to the total resistance to the flow of water in the shoot.

Since, from equations 2 and 6

$$K' = K.A$$

then, by assuming a nominal area for the xylem in the stems of Rhododendron shoots, it is possible to estimate K for the stems of these shoots for comparison with that obtained using stem segments. The area of the xylem at the apical ends of segments of one year old stems used in section 4.4 ranged from 2 - 10 x 10^{-6} m^2 with a typical area of around 5 x 10^{-6} m^2. Dividing K' of slightly stressed shoots (maximum value about 5 x 10^{-19} m^4) therefore gives a K value,

for comparison with that of stem segments, of about 1 x 10^{-13} m^2 with a range from 0.5 to 2.5 x 10^{-13} m^2.

Permeabilities of shoots calculated in this way are about 25% of those of stem segments. This argues strongly that a large proportion (about 75% if the values for K' of shoots are about right) of the resistance to water uptake by the water stressed cells of a shoot is between the xylem of the stem and the cells themselves. This is a much greater proportion of the total resistance than the 30% estimated by Tyree et al. (1975) to lie between the xylem and cells of Tsuga shoots. The discrepancy may be due to faults in calculating K' or to a very much lower resistance to flow in the vessels of Rhododendron than in the tracheids of Tsuga.

The failure of the permeability of water stressed shoots to recover when rehydrated was in contrast to acoustic (section 3.6) and permeability (section 4.4) experiments which showed that rehydration did refill the xylem conduits and restore xylem permeability.

If the failure to recover permeability is not an artifact it may be evidence that cavitation emboli in stems are not completely dissolved by overnight rehydration. Such partially dissolved emboli would expand to occupy and block the whole xylem conduit when the shoot was subjected to slight water stress prior to mounting on the potometers. Such reduced emboli would not expand in stem segments in which permeability was measured using water at near atmospheric pressure. However it is difficult to see why the xylem conduits of the leaves would not also remain unfilled in such a case. Clarification of this problem may require measurement of water flow through shoots under steady state conditions along the lines of experiments conducted by Hammel (1967) and Landsberg et al. (1976).

4.6. Cavitation and maintenance of plant water status

4.6.1. Introduction

The effects of cavitation on the transport of sap in the xylem should ideally be investigated in intact plants. In this way artifacts caused by cavitation occurring as samples are prepared for measurements are avoided. Moreover, in the absence of root pressure, cavitation emboli will be unlikely to dissolve (Zimmermann and Brown, 1971).

Assessing the effects of cavitation on sap transport in whole plants requires accurate measurements of water fluxes and pressure gradients in the xylem. Such measurements are often difficult to make. However, the effect of cavitation on sap transport in intact plants could be observed qualitatively by assessing the ability of the xylem to supply sufficient water to maintain transpiration and water potentials of the plants.

In the experiments described below two indicators of plant water status, water potential (Ψ) and stomatal conductance (g_s) were measured in potted Rhododendron plants which had been a) maintained on a normal watering schedule, b) denied water so that sap tensions high enough to cause cavitation developed, or c) denied water (so that cavitating sap tensions were developed) and then rewatered for up to five days.

It was expected that extensive cavitation would i) result in lower leaf water potentials in cavitated than in uncavitated plants if stomatal conductance was unaltered or that ii) water potentials would be maintained at the expense of transpiration (stomatal conductance would be reduced).

4.6.2. Ψ_l and g_s in uncavitated and cavitated Rhododendron plants

Potted Rhododendron plants in the glasshouse were stressed by withholding water for up to five weeks. Diurnal cycling of balance pressure was absent from plants which had been denied water for more than a few days. Stomatal conductances of these plants were too low to be measured.

After stressing to cavitating sap tensions (balance pressure > 1.9 MPa),

plants were rewatered and kept at near field capacity by twice daily waterings for three or five days before an experiment in which leaf balance pressure and stomatal conductance were measured at intervals over an entire day.

Leaf balance pressures were measured on four leaves from each plant at each sampling. Two of these leaves had been enclosed in small plastic bags and covered with aluminium foil for about an hour before sampling. This procedure allowed Ψ_l to equilibrate with the water potential of the xylem sap (Ψ_x) (Turner and Long, 1980). As the osmotic potential of xylem sap was known to be near zero (section 3.4.3) the balance pressures of the uncovered leaves were equated with leaf water potentials.

Stomatal conductance was measured on three leaves.

Mean values of leaf balance pressure and stomatal conductance for each plant during the day are shown in figure 49. This figure also shows the courses of relative humidity (RH), temperature (T) and incident radiation for the day. Highest night-time water potentials of all three treatments occurred just before dawn and were between 0.05 and 0 MPa. Exudation of sap, indicating night-time root pressures, was never observed. Similarly all plants exhibited minimum Ψ_l immediately before the plants were watered for the first time (at 1100 hours BST (British Summer Time)). Ψ_l was seldom more than 0.1 MPa less than Ψ_x during the daylight hours. Ψ_x and Ψ_l equilibrated quickly as darkness fell.

Minimum Ψ_x (nearly -0.6 MPa) and Ψ_l (-0.8 MPa) was found in plants which had not previously been subjected to water stress. Plants which had been stressed and rewatered three days earlier showed the least decline in Ψ_l and Ψ_x (0.3 MPa) during the day. Decreases in Ψ_l and Ψ_x in plants rewatered for five days were intermediate between those of the other two groups. The smallest differences between Ψ_l and Ψ_x were found in those plants which had been rehydrated for the shortest period. In these plants Ψ_l and Ψ_x were usually within 0.02 MPa of each other even when stomatal conductance was highest and the conditions for transpiration (low RH, high temperature) were most favourable.

Figure 49. Course of leaf balance pressures (P) and stomatal conductance (g_s) of potted Rhododendron plants during 8-9 July 1982.
Leaf balance pressures were used to measure leaf water potential (open symbol) and, by preventing transpiration, xylem turgor (closed symbols). Similar lines refer to the same plants in measurements of stomatal conductance and balance pressure.

NC = Plant maintained on normal watering schedule.

CR5 = Plants cavitated by stressing to sap tensions above 1.9 MPa, rewatered for 5 days.

CR3 = Plants cavitated by stressing to sap tensions above 1.9 MPa, rewatered for 4 days.

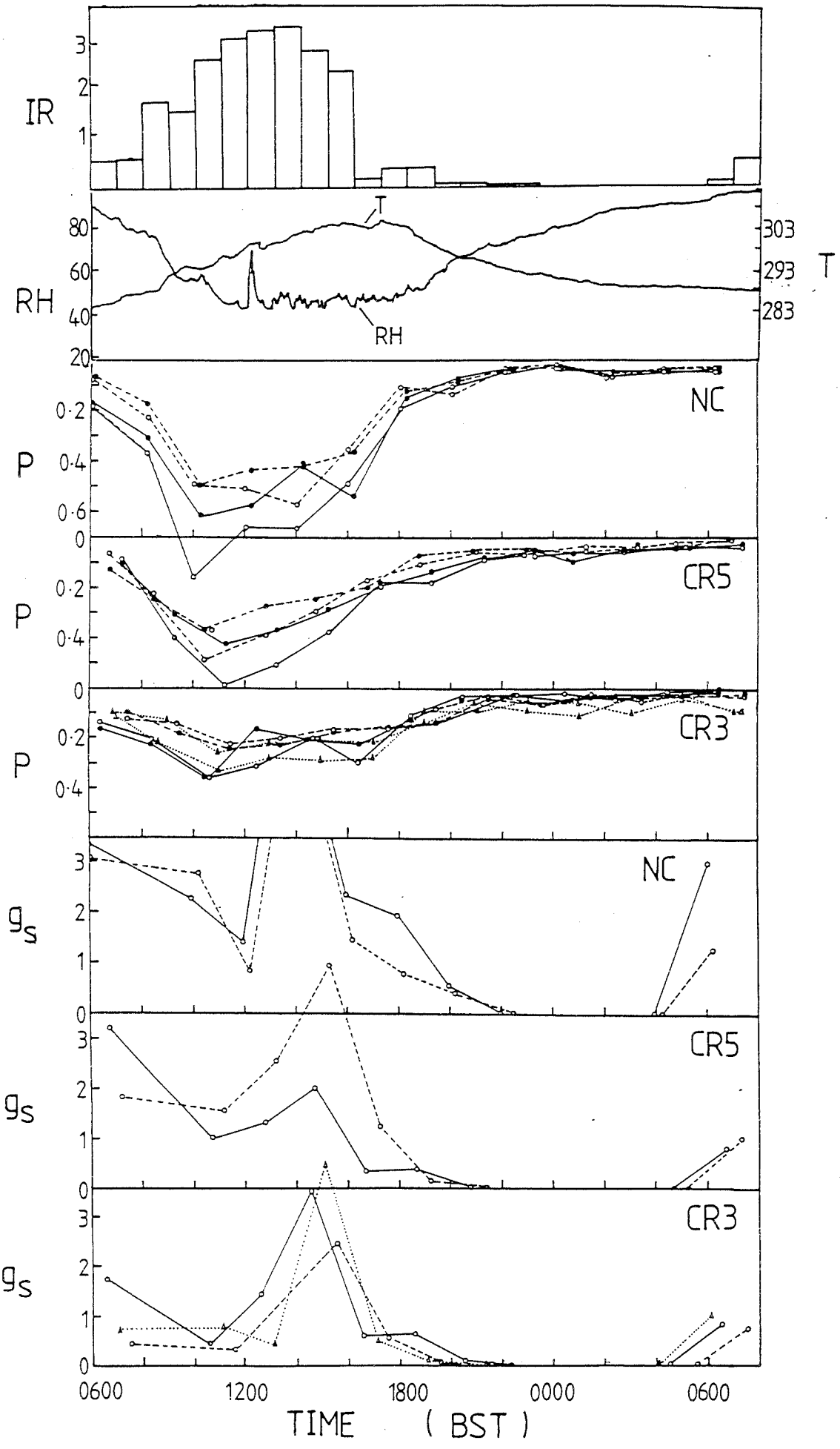
g_s = stomatal conductance ($m\ s^{-1} \times 10^2$)

IR = Incident radiation ($MJm^{-2}h^{-1}$)

P = Balance pressure (MPa)

RH = Relative humidity (%)

T = Temperature (°K)


IR
3
2
1
RH
80
60
40
20
T
RH
303
293
283
T
P
0·2
0·4
0·6
0
NC
CR5
CR3
g_s
3
2
1
0
0600
1200
1800
0000
0600
TIME (BST)

Stomatal conductance was too low to be measured during the hours of darkness but increased quickly at dawn. Stomatal conductance of all plants declined from the time of the first measurement (just after 0600 hours) until the plants were rewatered at 1100 hours after which marked rises in stomatal conductance were observed.

The highest stomatal conductances and the greatest response to watering were found in those plants which had not previously been subjected to water stress. The lowest stomatal conductances were found in plants which had been highly stressed and rewatered for only three days. Plants which had been stressed and rewatered for five days were intermediate in response between those which had not been stressed and those which had been stressed and rewatered for three days.

4.6.3. Permeability of stems cut from stressed whole plants

Stem segments 100 to 150 mm long were cut from one or two year old shoots of the potted Rhododendron plants the day after the all-day experiment described above. The results of these experiments are given in figure 50. The permeability of shoots from plants which had not experienced sap tensions of over 1.8 MPa were comparable to those of uncavitated stems of similar age cut from the bush in the laboratory grounds (table 13).

K was found to be lower in stems from plants of sap tensions greater than 1.8 MPa than in plants of lower sap tensions. K was lower still in stems sampled from plants which had been subjected to high sap tensions and then rewatered for four days.

4.6.4. Discussion

The original aim of this experiment, to assess the effect of cavitation on sap transport in intact plants, was not achieved. This was because after-effects of water stress modified the response of stomata to plant water status. This after-effect limited maximum stomatal conductances for several days after

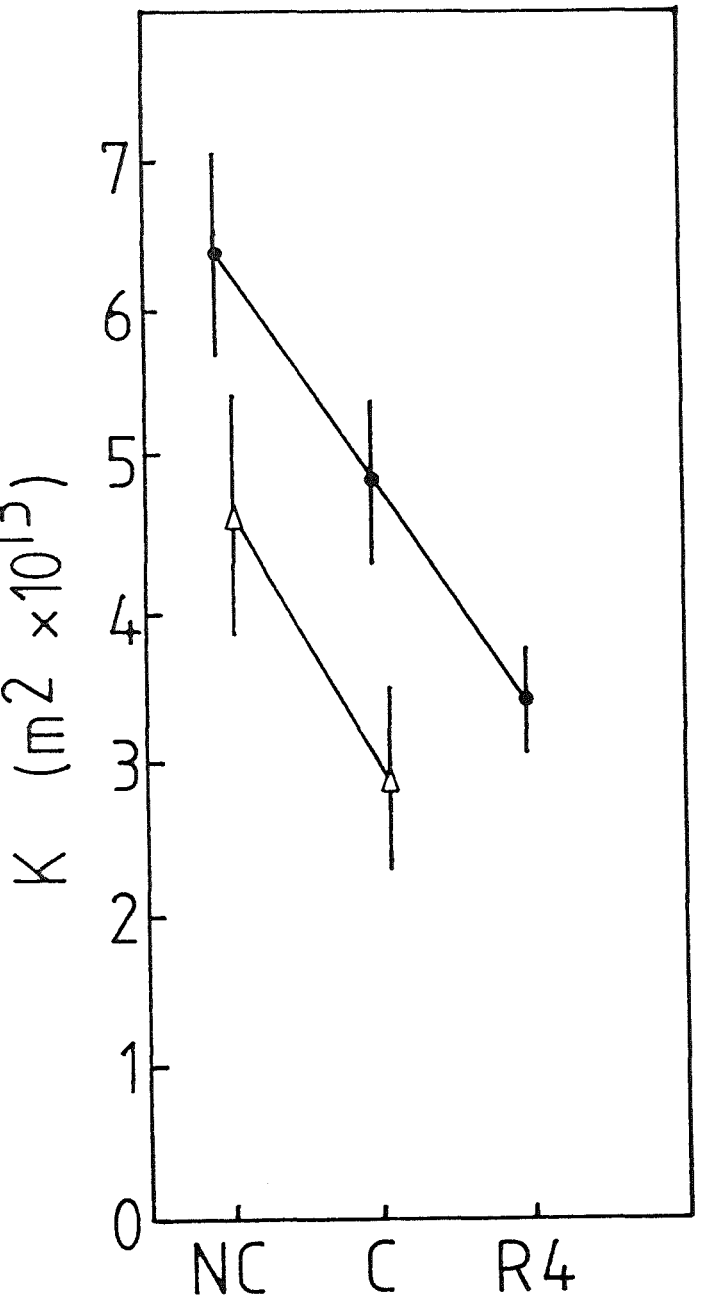


Figure 50. The effect of water stressing of whole Rhododendron plants on the permeability of stems. Stem segments were cut at intervals as sap tensions increased and four days after the plants had been rewatered.

NC - Not cavitated (balance pressure < 1.9 MPa)

C - Cavitated (" " > 1.9 MPa)

R4 - Four days after plants were rewatered (minimum daily balance pressure = 0 - 0.5 MPa)

Closed symbols = Relative conductivity calculated from flow into stem segments.

Open symbols = Relative conductivity calculated from flow out of stem segments.

the plants had been rewatered.

The after-effect of stress and the time taken for it to disappear (five days or more) were similar to the results of experiments with Nicotiana and Vicia which had been subjected to severe water stress and re-supplied with water (Fischer et al., 1970). It has been shown that most of the prolonged reduction in stomatal aperture is due to changes in the guard cells themselves (Fischer 1970) and is not caused by decreased permeability of the xylem.

Without concurrent measurements of stomatal conductance, transpiration and water potential it is therefore impossible to identify short-term decreases in xylem conductivity from the results of experiments of this type. However the similarity of Ψ_1 and stomatal conductance of plants rewatered for five days to those of the unstressed plants is an indication that the ability of the xylem to supply the water requirements of the leaves in the long term was not seriously impaired by the period of water stress. This is in agreement with the observation that even the most severely wilted plants quickly recovered when rewatered and subsequently appeared similar to plants which had not been stressed.

Measurements had indicated that high sap tensions had reduced stem permeability to 20 - 30% below that of similar stems from unstressed plants. A similar reduction in permeability was also found after the stressed plants had been rewatered for several days (figure 50).

The apparent contradiction of the measurements of Ψ and stomatal conductance (which showed that the xylem was still able to supply the water requirements of the leaves) by the results of the permeability experiments may be reconciled by remembering that a) cavitation occurs progressively over a range of increasing sap tensions (section 3.5) and b) high levels of redundancy are usual in the supply of xylem conduits (e.g. Mackay and Weatherly, 1973; Zimmermann and Brown, 1971).

The failure of the permeability of stem segments to recover when the water stressed plants were rewatered was in agreement with the findings that the ability of the leaves to produce clicks was not recovered either (section 3.6).

That recovery might not have been possible because of failure of sap pressure to increase sufficiently for xylem emboli to dissolve has already been discussed (section 3.6).

The cause of the continued decline in permeability of stems when water stressed plants had been rewatered was unknown. Because of alterations to the permeability apparatus it was only possible to measure the flux of water entering these stems (Q_{in}). As these rewatered stems were unlikely to take in much water to relieve internal water deficits (section 4.4) Q_{in} may have been low relative to Q_{in} of shoots of the same permeability but higher water deficits. However such differences have been shown to be negligible in the stem segments of this size so that the continued downward trend in permeability is probably correct.

It is possible, although unlikely, that the decrease in permeability was the result of tylose invasion of cavitated xylem conduits. Previous observations had shown that tyloses did not develop in Rhododendron shoots detached from the plant (section 4.4).

4.7. Summary of chapter 4.

The effect of high sap tensions on the permeability of xylem of Rhododendron was investigated using stem segments, shoots or whole plants. Decreases in the area of the xylem conducting sap and in the permeability of the xylem were found as sap tensions increased. It is proposed that these changes were due to progressive cavitation of xylem conduits occurring as sap tensions increased above about 1 MPa.

Chapter 5. Exclusion of gas from conduits by pit membranes

5.1 Introduction

A fundamental part of the cohesion theory is that gas is prevented from entering sap-filled xylem conduits by sap retained in the small pores of the pit membranes by capillarity (Dixon and Joly, 1894). This has been discussed in section 1.5.2.

It has been suggested that differences in the size of pores in pit membranes may be a factor determining the susceptibility of different species to wilt diseases (Van Allen and Turner, 1975). If such differences in pore size do exist they may also have a role in determining the pressure differentials at which gas may cross wet pit membranes and enter xylem conduits. The pressure differential required to expel sap from the pores of the pit membrane depends upon the radius of the pore and the surface tension of the sap. Air will therefore penetrate the pit membranes irrespective of whether the pressure differential is established by high external gas pressure or high internal sap tension. The pressure at which the conduit becomes gas filled depends only on the size of the largest pore in the membrane as any gas entering by this pore will immediately expand to occupy the entire conduit (section 3.6).

The experiments reported below were carried out to determine the pressure differential required to force sap from the pores of the pit membranes of Rhododendron stems and to relate this to the sap tension at which cavitation occurs in the same species.

The work was in three parts:

5.2) Determining the pressure at which gas first passes through a segment of stem.

5.3) Gas permeability of stems as an indicator of the pressure required to displace sap from the pores of pit membranes.

5.4) The expression of sap from stems as an indicator of the pressure required

to displace sap from the pores of the pit membranes.

5.2. First passage of gas through stems

Nitrogen gas could be passed through 200-400 mm long Rhododendron stems at pressures of less than 0.1 MPa. This was shown by mounting the stems in the pressure chamber and collecting the gas emerging from the end of the stem outside the pressure chamber under water.

Passage of gas at such low pressures indicates that some xylem conduits extended over even the longest stems used (Greenidge, 1955a; Zimmermann and Jeje, 1981). These experiments indicate that vessels in Rhododendron stems were longer than the 110 mm indicated by injection of Indian ink (section 4.2).

As the maximum vessel length in Rhododendron stems was greater than that of stems suitable for mounting in the pressure chamber this simple type of experiment could not be used to find the pressure required to force sap from the pores of the pit membranes.

5.3. Permeability of stems to gas as an indicator of the pressure required to displace sap from the pores of pit membranes

5.3.1. Introduction

Progressive expulsion of sap from the pores of pit membranes will open more and more paths to gas flow and increase flow of gas through the stem (Yao and Stamm, 1967).

Moreover, it has been shown that a linear relationship exists between the quotient of the gas flux $Q(m^3s^{-1})$ divided by the pressure driving gas flow, ΔP, (i.e. $Q/\Delta P$) and ΔP when gas is forced through an unchanging, homogeneous resistance such as a ceramic plate (Adzumi, 1937) or a microporous filter membrane (Yao and Stamm, 1967). When gas flows through a complex series of resistances, such as is comprised by the pit membranes and tracheid lumina of

Abies (Petty and Puritch, 1970) or several woods laid together (Smith and Banks, 1971), gas flow will be complexly determined by the sum of the resistances in the series. However, at pressures above about 0.05 MPa one of the resistances will usually dominate and $Q/\Delta P$ will appear to be linearly related to ΔP (e.g. Smith and Banks, 1971).

As expulsion of sap from the pores of the pit membranes will increase the permeability of the stem to gas $Q/\Delta P$ will increase more rapidly with increases in ΔP than would be the case if the permeability of the segment had remained unchanged.

A curvilinear rather than linear relationship would therefore exist between $Q/\Delta P$ and ΔP. Such a curvilinear relationship between $Q/\Delta P$ and ΔP will be observed irrespective of whether or not some vessels in the stem segment are initially open across the whole length of the segment.

Section 5.3.2. Permeability of Rhododendron stems to gas

Figures 51 and 52 show the results (expressed as the relationship between $Q/\Delta P$ and ΔP) of experiments in which the flow of nitrogen gas through Rhododendron stems was measured over one or more cycles of stepwise increases and decreases in pressure between 0 and 4.14 MPa. Each stem was usually subjected to two pressure cycles.

The hysteresis in the relation of $Q/\Delta P$ to ΔP, with $Q/\Delta P$ being higher when ΔP was falling than when increasing for the first time, was found in all twenty-three experiments of this type. $Q/\Delta P$ increased less on a second pressure cycle than on the first and the relation of $Q/\Delta P$ to ΔP, instead of being markedly curvilinear as on the first pressure cycle, was often nearly linear (e.g. figure 52).

On eight occasions stems which had been used in the above experiments were rehydrated by soaking them overnight in distilled water. The flow of gas through the rehydrated stems was then measured (figure 51). Rehydration returned $Q/\Delta P$ to values similar to those found on the first cycle of pressure.

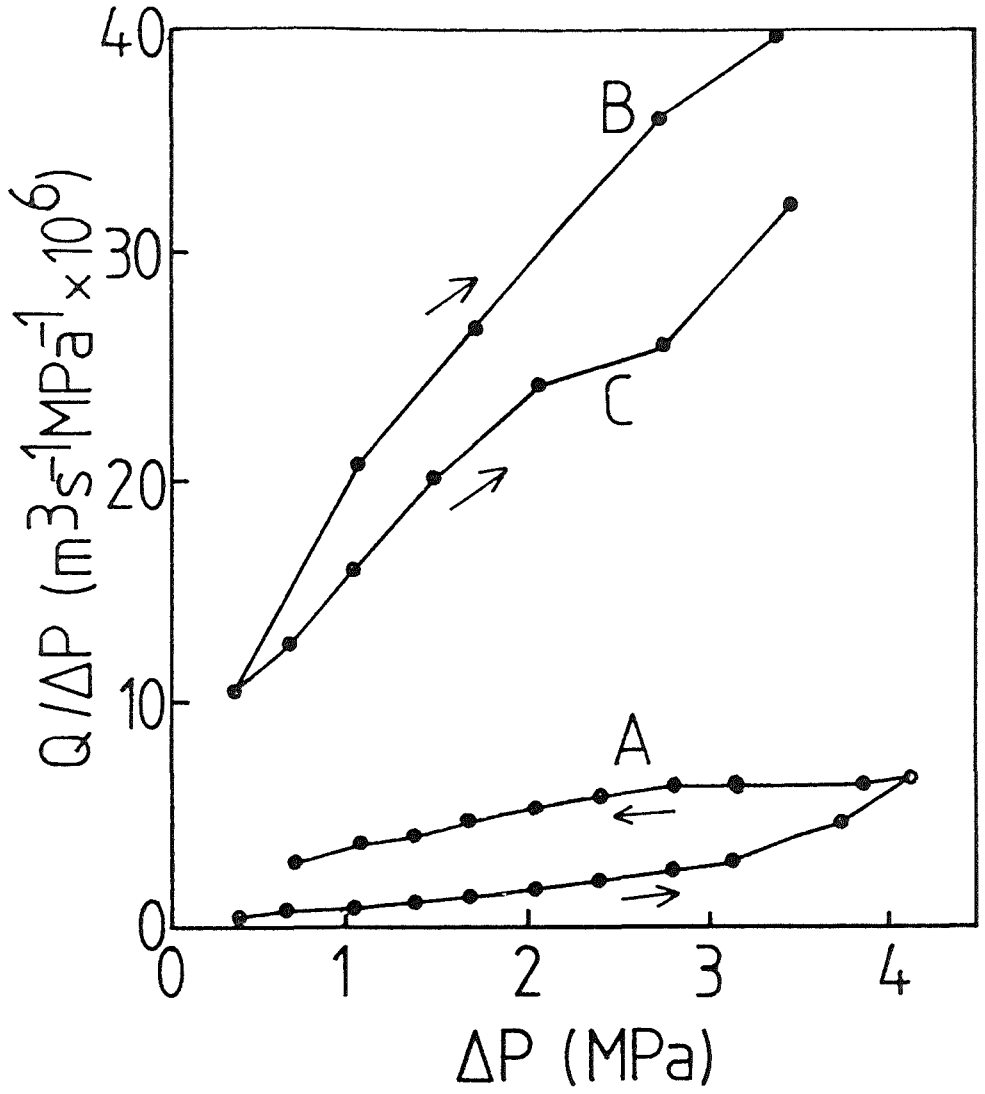


Figure 51. The flow of gas through a Rhododendron stem.

A - Fresh stem. (Arrows indicate whether pressure is rising or falling.)

B - After drying in air.

C - After oven drying at 90°C.

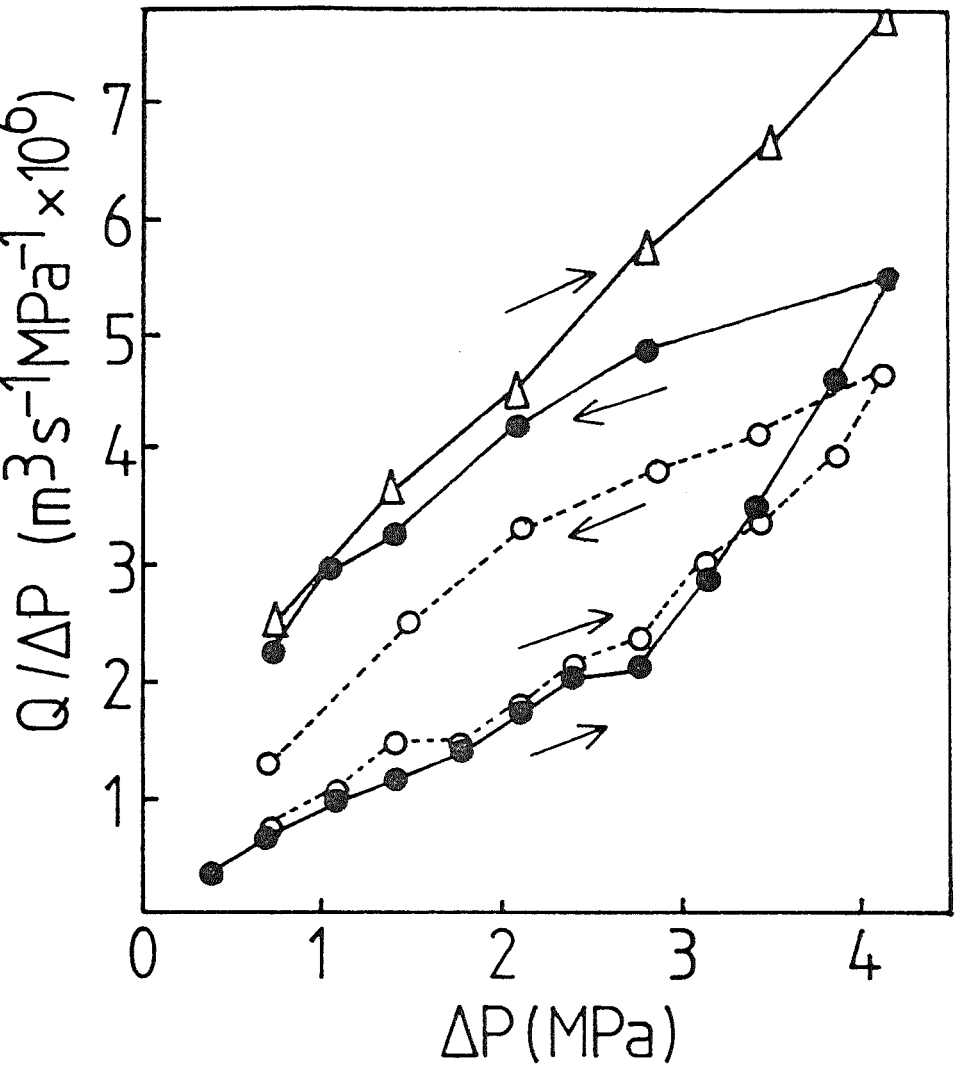


Figure 52. The flow of gas through a Rhododendron stem.
The arrows indicate when pressure is increasing or decreasing.

o First pressure cycle with fresh twig.

Δ Second " " " " "

o First pressure cycle after overnight rehydration.

When ΔP was decreasing in the second half of the pressure cycle $Q/\Delta P$ was sometimes, as in figure 52, slightly less than when the flow of gas through the stem was measured for the first time. This was attributed to contamination of the xylem, probably by bacteria, occurring during rehydration.

5.3.3. Discussion

The curvilinear relation of $Q/\Delta P$ to ΔP and the hysteresis found on a first pressure cycle is evidence that the permeability of the stems to gas increased as pressure increased. As $Q/\Delta P$ does not fall to its initial value when pressure is reduced, it is unlikely that the increase in $Q/\Delta P$ was the result of elastic deformation of structures within the stem. Also the reduction of $Q/\Delta P$ to its original values by rehydration (figure 52) shows that the increase in the gas permeability of the stem was not the result of mechanical damage, including elastic deformation and rupture of pit membranes, occurring during the first cycle of increasing pressure.

The curvilinear relationship of $Q/\Delta P$ to ΔP on the first cycle of increasing pressure is consistent with the hypothesis that sap is progressively expelled from pit membrane pores of smaller diameter as pressure is increased. The hysteresis in the relation of $Q/\Delta P$ to ΔP indicates that these pores do not refill as pressure falls. In addition, the smaller increases in $Q/\Delta P$ and the near linear relation of $Q/\Delta P$ to ΔP found on a second pressure cycle (figure 52), indicate that most pit membrane pores too large to retain sap against the applied pressure are emptied on the first pressure cycle.

The linear relationship of $Q/\Delta P$ to ΔP found when using air or oven-dried stems (figure 52) and in some moist stems on a second pressure cycle (figure 51), is evidence that, as assumed when designing this experiment, viscous flow of gas occurs over the range of pressures used in this experiment. Moreover, it shows that if the permeability of the stem to gas flow is unchanging, even though composed of several distinct resistances in combination, a linear relation of $Q/\Delta P$ to ΔP is to be expected over the range of pressures used in these experiments and as shown by Smith and Banks (1971). A curvilinear relation of the type found in figures 51 and 52, therefore, indicates a change in the permeability of

the stems.

The increased gas permeability of the stems may have been due in part to evaporation of water from the stems by the gas passing through them. Although the rate of gas flow through a stem held at a pressure of 1.4 MPa did not increase over a two hour period, increases in the rate of flow with time were often found at higher pressures. It was thought that these increases were due to evaporation of sap from the angular crevices formed where fibrils overlie each other at the edges of the pit membrane pores. Although such evaporation will be unlikely to open new pores to gas flow it will increase the area available to flow in those already open, and thereby increase the flow of gas at a given pressure (Stamm, 1935).

Because of evaporation at high pressures the hysteresis in $Q/\Delta P$ with changes in ΔP, although indicating that sap is forced from the pores of the pit membranes, cannot be used to determine the sizes of these pores. Such an analysis would be nearly impossible even in the absence of evaporation, as pores will probably vary in size both within and between individual pit membranes.

5.4. Expression of sap from stems by gas under pressure

5.4.1. Introduction

Expulsion of sap from the pores of pit membranes will admit gas to the conduit behind the membrane. Because the gas is under pressure any sap in the conduit will be forced from it, either to be taken into other partly filled conduits or water-stressed cells, or to be expressed from the low pressure end of the stem. Measurement of the gas pressure at which sap is first expressed from the end of a stem mounted in the pressure chamber will therefore enable calculation of the size of the largest pores in the pit membranes.

This method of calculating the sizes of the pores is independent of measurements of gas flow through the stems. In addition, because all the sap in the conduit is expelled when the first gas enters, continued expression of sap at successively higher gas pressures will indicate the existence of conduits

bounded by pit membranes of successively smaller pore sizes.

The role of capillarity in determining the pressure at which sap will be forced from pit membranes can be shown by altering the surface tension of the sap in the stems. The pressure needed to displace sap from the capillaries of the pit membranes will, if equation 10 holds, be proportional to the surface tension of the liquid in those capillaries.

5.4.2. The pressure at which sap is expressed from stems

a) After transpiring water

Stems from shoots brought to full turgor by uptake of distilled water were mounted in the pressure chamber. The sap expressed after successive pressure increments was collected by absorbing onto tissue paper in plastic phials (section 2.6.2).

The collections of sap made after each pressure increment, as pressure increased from 0 to 4.14 MPa, are shown in figure 53a.

In four of five experiments a major expression of sap occurred as pressure rose from 1.38 to 1.73 MPa. In the fifth experiment of this series the expression began at chamber pressures between 1.03 and 1.38 MPa. In three experiments this phase of the expression had become minimal or ceased at chamber pressure of 3.5 MPa, and in the remaining two experiments had begun to decline by this pressure.

In subsequent experiments (figure 53b,c,d) the accuracy of the estimation of the pressure at which the first expression of sap occurred was improved by reducing pressure increments from 0.34 MPa (50 psi) to 0.17 MPa (25 psi) at pressures between 0 and 2 MPa.

In two of the experiments shown in figure 53a, sap was also collected in relatively large volumes over the first 0.34 or 0.69 MPa of applied pressure. This expression occurred over a narrow range of pressures and had declined to near zero by the pressure at which the major phase of exudation began. This

Figure 53. The expression of sap from Rhododendron stems. Histograms show sap collections after each pressure increment as a percentage of the total expression for the shoot. In instances where pressure increments were reduced from 0.34 to 0.17 MPa the expressions over small increments are shown in black.

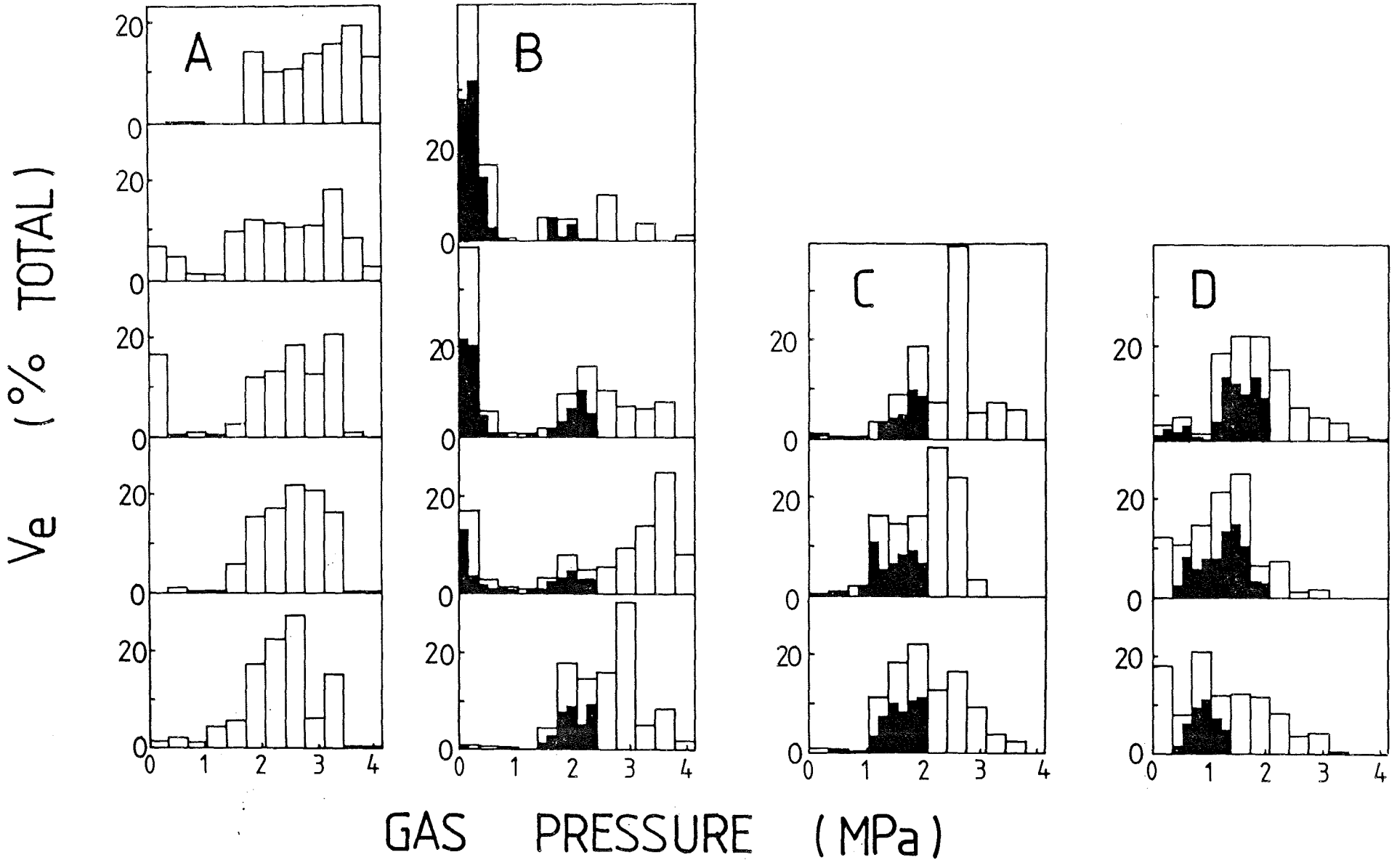
Experiments were made after shoots had been taking up:

A. Distilled water ($\sigma = 71 \times 10^{-3}$ Pa m)

B. 1 Molal NaCl ($= 74 \times 10^{-3}$ Pa m)

C. 4% N-butanol ($= 48 \times 10^{-3}$ Pa m)

D. 10% N-butanol ($= 27 \times 10^{-3}$ Pa m)


A
B
C
D
Ve (% TOTAL)
GAS PRESSURE (MPa)
0
20
0 1 2 3 4

first expression was thought to be of sap from xylem conduits which had been opened when the stem had been cut. Because of the relatively large diameters of the lumina of the xylem conduits little gas pressure is required to overcome capillarity and to force the sap from them.

This first, brief expression is also seen in many other experiments (figure 53b,c,d). Slight differences in stem size and preparative technique were believed to be the cause of the differences in the size of this first expression from different shoots. Allowing air to enter the cut end of transpiring shoots for several minutes before removing the stem for experiments will tend to remove sap from conduits open at the basal end of the stem and result in a smaller initial expression of sap.

b) After transpiring a strong osmotic solution

Two explanations for the expulsion of sap at chamber pressures of 1.4-1.7 MPa were considered. One, that gas was penetrating pit membranes at these pressures, has already been discussed (section 5.4.1).

However, it was also possible that the major phase of sap expression was due to a pressure-volume curve type of expression from cells in the stem subjected to increasing gas pressure (Scholander et al., 1964; Tyree and Hammel, 1972). If this is the case, then the largest volume of sap to be expressed by an increment in chamber pressure will be found close to the pressure at which cell turgor is reduced to zero, i.e. the point of incipient plasmolysis.

It is known from balance pressure data that incipient plasmolysis in Rhododendron leaves occurs at leaf balance pressures of about 1.7 MPa (section 3.4.4, table 7). The expression of sap from stems at chamber pressures of 1.4-1.7 MPa might therefore represent the first large expression of sap from cells which have lost their turgor.

These two possibilities can be separated by reducing cell turgor to zero before applying gas under pressure to the stems.

Cell turgor was reduced to zero by setting the stems to transpire 1 M NaCl solution (Ψ_s = -4.37 MPa) for three hours before cutting the stem for the

experiment. As the surface tension of a 1 ml NaCl solution (74.4×10^{-3} Pa m^{-1} at 298°K) is not very different to that of water at the same temperature (71.2×10^{-3} Pa m^{-1}) the gas pressure required to express sap from the capillaries of pit membranes is virtually unaffected by substituting the salt solution for distilled water in the transpiration stream. However, as cell turgor is zero at this water potential (table 7) sap will be expressed in large volumes from cells as soon as gas pressure is applied. The expression beginning at 1.4 - 1.7 MPa would be absent.

That the living cells of the stems had lost their turgor after three hours of transpiring the salt solution was tested for by measuring Ψ_l of leaves from the shoots. In addition, the osmotic potential of sap expressed from these stems was also measured. Both Ψ_l and Ψ_s were measured using the thermocouple psychrometers. The results are presented in table 16.

Table 16. Ψ_l and Ψ_s of leaf discs and xylem sap respectively from Rhododendron shoots which have been transpiring 1 ml NaCl solution (Ψ_s = -4.37 MPa) on a laboratory bench for three hours. Sap was collected by applying pressure to one end of stem segments mounted in the pressure chamber. The pressures applied to make the expressions are indicated in the table.

Shoot	Ψ_l (MPa)	Ψ_s xylem sap	
		Pressure applied to make sap collection from stems	
		0 - 0.34 (MPa)	1.38 - 1.72 (MPa)
1	- 0.45	-	-
2	- 0.86	-	-
3	- 0.57	- 1.60	- 2.08
4	- 1.44	- 3.24	- 2.67

As can be seen, neither Ψ_l nor sap Ψ_s fell to that of the salt solution. This was probably the result of dilution of the transpired salt solution by water

already present in the leaves and xylem.

However, sap Ψ_s was generally lower than that by which Rhododendron leaves have lost all turgor (about -1.7 MPa). Assuming that the cells of the xylem are nearly in equilibrium with the osmotic potential of the xylem sap, Ψ of the living cells of the stem has been reduced sufficiently to eliminate turgor.

The results of expression experiments using stems from shoots prepared similarly to those of table 16 are presented in figure 53b.

The expression of sap beginning as chamber pressure rose from 1.4 - 1.7 MPa was found in all four experiments.

Because of the large initial expression in three of the four stems of this experiment, the possibility that sap expression was linked to the loss of turgor by these stems cannot be completely discounted. However, it was thought that sap was not expressed from stems as the result of turgor. This was because

i) in all four experiments shown in figure 53b sap was expressed over a range of pressures, beginning at 1.4 to 1.7 MPa, similar to that found in stems which had been transpiring distilled water.

ii) Some stems had little or no expression of sap at low pressure.

iii) Sap expulsion from stems ceased within eight minutes of the increment in pressure. This was much shorter than would have been expected if sap had been expressed from cells (e.g. Tyree and Dainty, 1973).

Moreover, the length of stem inside the chamber was deliberately kept short to minimize the effect of chamber pressure on the living tissues of the stem.

c) After transpiring solutions of reduced surface tension

The surface tension of sap in the stems of Rhododendron shoots was lowered using aqueous solutions of N-butanol (table 1). The solutions were infiltrated into the xylem by supplying them to the cut ends of shoots transpiring on the laboratory bench. It was assumed that the N-butanol solutions had occupied the lumina of the conducting xylem conduits after three hours of transpiration.

The results of expression experiments using stems supplied with aqueous butanol solutions of 10% (V/V) (surface tension = 27 Pa m x 10^{-3}) or 4% (V/V) (surface tension = 48 Pa m x 10^{-3}) are shown in figures 53c and 53d respectively.

In both cases the pressure at which the major expression of sap occurred was reduced compared to shoots which had transpired only distilled water.

When 4% N-butanol solution had been supplied, the major phase of sap expression began at chamber pressures between 1.03 and 1.21 MPa in two cases and 0.86 and 1.03 MPa in the third. These pressures are approximately $^{2}/3$ of those at which sap is expressed from stems containing distilled water.

When 10% N-butanol (i.e. saturated aqueous solution) had been supplied, sap expression began at around 0.5 MPa in two stems and 1 MPa in the third. The pressure increments used were too large to define the pressure at which the first expression of sap occurred to an accuracy of more than ± 0.2 MPa. Thus reducing surface tension by nearly two-thirds (from 71 to 28 x 10^{-3} Pa m) also reduces by two-thirds the pressure required to force sap from the xylem (from 1.5 to 0.5 MPa).

5.4.3. Discussion

The aim of establishing the pressure differentials at which sap will be forced from the pores of the pit membranes of Rhododendron stems was successfully accomplished by noting the pressures at which sap was expressed from stems.

Sap was found to be expressed from the stems (from shoots which had been transpiring distilled water) over a range of pressures beginning at around 1.4 MPa. Although there was considerable variability in the pressure at which the last sap was expressed from the stems, expression had fallen to very low levels, or started to decline, at chamber pressures of around 3.5 MPa.

This is a similar range of pressure differentials across pit membranes to those at which clicks are detected in Rhododendron leaves (leaf balance pressures of 1.4 to 3 MPa, June 1980, table 9). It may therefore be possible that

cavitation detected in leaves by the acoustic method occurs as the result of gas entering xylem conduits from outside, rather than the formation of the gas phase within conduits. Such gas may be already present in stems in embolised conduits, probably mainly in the non-conducting fibres, but also in cavitated vessels, or it may enter stems through weather checks or other wounds in the wood.

The pressure at which sap is first expressed from the stems can be used to determine the radii of the pores of the pit membranes by application of equation 10 (section 3.6).

Equation 10. $$Pc = \frac{2\sigma \cos \theta}{r}$$

In this case the capillary pressure is equivalent to the gas pressure at which sap is forced from the pores of the pit membranes. However, the pores of the pit membrane are not circular, as they are actually the spaces between the cellulose fibrils of which the pit membrane is constructed. Therefore, equation 10 can only be used to obtain an equivalent radius of the actual pore dimensions (Petty, 1972).

Sap is first expressed from stems at gas pressures of about 1.4 MPa.

Rearranging equation 10 and substituting 1.4 MPa for Pc and 71×10^{-3} Pa m for σ,

$$r = \frac{142 \times 10^{-3}}{1.4 \times 10^{6}}$$

$$= 100 \text{ nm}$$

Similarly, from the pressure at which the last pit membranes are penetrated by gas, ie 3.5 MPa, the largest pores in these membranes have a radius of 41 nm. The largest pores of this range are about the same size as the pores in bordered pits of Abies (Petty and Puritch, 1970) and slightly smaller than in the heartwood of a number of other conifers (Stamm, 1929). The largest pores in the pit membranes with the smallest pore size (about 40 nm) are less than half that of the mean in several conifers. These calculated radii are for the largest pores in the pit membranes. Most pores may be much smaller than this (Stamm, 1929).

These estimates of membrane pore sizes were calculated on the assumption that retention of sap in these pores can be adequately described by equation 10. Experiments in which the surface tension of sap in these conduits was altered by adding N-butanol have shown the pressure required to force sap from the pit membrane to be proportional to the surface tension of the sap, as predicted by equation 10. The assumption that sap is retained in these pores by capillarity therefore seems justified.

Section 5.5. Summary of Chapter 5.

The pressure required to force sap from the pores of the pit membranes of Rhododendron stem was investigated. Gas was found to penetrate the pores of the pit membranes at pressures of about 1.4 - 3.5 MPa. These are similar to those at which cavitation occurs in Rhododendron, and it is suggested that cavitation in detached leaves and shoots of this species occurs when gas penetrates the pit membranes. The increase in xylem permeability caused by high gas pressures is reversible and therefore not due to mechanical damage to xylem conduits by high gas pressures.

Chapter 6. General Discussion

The results of the experiments described in Chapters 3 to 5 may be used to attempt to answer the three problems stated at the beginning of this thesis: i) are clicks caused by cavitation of xylem sap and, if so, at what sap tension does it occur, ii) does cavitation affect pressure chamber measurements of plant water potential and iii) what is the effect of cavitation on the flow of sap in the xylem. In addition, a further question may be answered: how does the gas phase arise in the cavitating conduit?

New evidence that acoustically detected clicks are the result of cavitation was obtained in two experiments. In the first the appearance of gas in Acer stems was co-incident with the detection of clicks (section 3.2.7). In the second, the production of clicks by Rhododendron leaves was shown to be dependent on sap tension and to be independent of cell turgor (section 3.5.3). This new evidence complements the circumstantial links between cavitation and i) increases in sap tension and rehydration (Milburn and Johnson, 1966; Milburn, 1973a,b; Milburn and McLaughlin, 1974), ii) the dehiscence of fern sporangia (Milburn and Johnson, 1966; Stocker, 1952) and iii) changes in the rate of water uptake by Ricinus leaves (Milburn, 1966) which have been obtained previously. Although a 'click' has yet to be directly associated with the occurrence of a bubble in a xylem conduit it now seems well established that clicks are caused by cavitation of sap in xylem conduits. Louder clicks were found in woody samples than in more succulent ones. This is believed to be because of better conduction of vibrations in hard, woody tissues than in soft ones. However clicks produced at higher sap tensions (as in trees) might also be expected to be louder than those produced at lower sap tensions (as in herbs) because of the greater energy stored in the deformed walls of the conduits containing sap under high tensions than low.

Cavitation was not found to affect pressure chamber measurements of water potential in Rhododendron leaves (section 3.4.3). However differences between balance pressures of Rhododendron shoots and leaves which became apparent as

leaf balance pressures rose above 2 MPs were tentatively attributed to cavitation (section 3.4.2). These results are in contrast to those for Malus leaves (West and Gaff, 1971) and Rhododendron roseum shoots (Boyer, 1967) but are similar to those obtained with many other species (Ritchie and Hinckley, 1975). It is thought that cavitation of xylem is unlikely to have measurable effects on balance pressure if the xylem contains only a small fraction of the total water content of the sample. This is probably the case in leaves but not in shoots, which have a large volume of xylem in the stem. The pressure chamber could therefore be used to determine the sap tensions in leaves during investigations of the relationship between cavitation and sap tension.

The sap tensions at which cavitation occurs in species of herbs, shrubs and trees were determined using the acoustic detector and the pressure chamber. From the results of these experiments cavitation profiles relating cavitation to sap tension were drawn up (section 3.5). The cavitation profiles of all species except Fraxinus were bell-shaped with most clicks being detected over a relatively narrow range of sap tensions. In general cavitation occurred at lower sap tensions in herbs than in trees (table 8). With the exception of Fraxinus clicks were not found at sap tensions above 3.5 MPa. Cavitation has been shown to occur at similar sap tensions in intact plants (table 18). The cavitating sap tensions determined using the acoustic detector and the pressure chamber were therefore though to be reliable estimates of the sap tensions causing cavitation in whole plants.

The sap tensions causing cavitation in the species used in this study are low by comparison to those at which cohesive failure of sap is to be expected on theoretical grounds (Oertli, 1971). In addition the apparently reliable experiments of Briggs (1955) showed cavitation of water in glass tubes to occur at tensions of about 30 MPa, ten times those found in this study. The comparatively low and, between species, variable sap tensions causing cavitation in plants also suggests that cavitation in the xylem is not the result of tensions exceeding the limits to cohesion within the sap. The limits to sap tension might be determined by differences in the construction of the xylem conduit of each species. The materials in the cell wall and the solutes in the sap itself

will determine the limits to adhesion between the sap and the conduit walls. The size of the pores of the pit membrane and walls will determine the sap tension at which gas can enter the conduit and the maximum size of any small bubbles entering as entrained nuclei on particles in the sap. Other aspects, such as the sculpturing and deformability of the conduit walls, may also be involved.

There are several reasons for suspecting that cavitation occurs when gas is drawn through the pit membranes and into a sap-filled conduit. Firstly, the experiments of chapter 5 have shown gas to penetrate the wet pit membranes of Rhododendron stems at about the same pressure differentials which exist when cavitation occurs (1 - 3.0 MPa). Moreoever the pressure at which gas penetrated the pit membranes varied in direct proportion to changes in the surface tension of the sap wetting the pit membranes. Both the results are expected if sap held in the pores of the pit membranes by surface tension was preventing the passage of gas. The report that clicks were heard when slightly stressed Ricinus leaves were supplied with ethanol (surface tension = 22.8 x 10^{-3} Pa m^{-1}) (Milburn, 1973a) is also consistent with this proposal. In intact plants there is likely to be enough gas in embolised fibres and ininterstitial spaces, entering the xylem through wounds or in cavitated conduits to initiate cavitation of neighbouring sap filled conduits in this way.

Differences in the sap tensions at which sap cavitates in different species, in young and mature xylem or even in single leaves, may be due to differences in the size of the pores in the pit membranes. Larger pores will allow gas to enter and cavitate the conduit at lower sap tensions than will smaller pores. Species-related differences in the size of the pit membrane pores have already been cited as a factor determining the susceptibility of different species to wilt diseases (Van Allen and Turner, 1975). The same differences might also determine the sap tension at which each cavitates.

The proposal that the tensions at which cavitation occurs are determined by differences in the structure of the pit membrane may be tested by repeating the experiments of chapter 5 with another species, for instance tomato, in

which cavitation occurs at a much lower sap tension than in Rhododendron.

The experiments of chapter 5 indicated that the largest pores in the pit membranes of Rhododendron were 0.2 to 0.08 μm in diameter. This is nearly the same size as has been found in many gymnosperms by the same technique (Stamm, 1929). If the scheme proposed above is correct gymnosperms will cavitate at the same sap tension as angiosperms with similar pit membrane pore sizes, despite occlusion of the pit annulus by the torus. What then is the function of the bordered pits of gymnosperms if not to prevent cavitation at high sap tension? The bordered pits may be an adaptation minimizing the risk of cavitation caused by freezing and not by high sap tensions. It has been proposed by Hammel (1967) that toral occlusion prevents the release of pressure in tracheids when the sap that they contain freezes. Upon thawing the bubble of gas forced out of solution by freezing is forced back into the solution by this pressure before the sap comes under tension. In the absence of the bubble cavitation cannot occur. Angiosperm vessels, which lack bordered pits, are cavitated by freezing (O'Malley, 1979).

However Sucoff (1969) has suggested that flow of water from some thawing conduits to other thawing conduits may delay the imposition of sap tensions in the latter long enough for bubbles to dissolve. The conduits losing water (estimated to be about 10% of the total number conducting sap before freezing) will be cavitated.

The effect of cavitation on the flow of sap in the xylem of Rhododendron was investigated in whole plants, shoots and segments of stem (chapter 4). The permeability of shoots and stem segments was reduced by 60% or more as sap tensions increased above 1 MPa. Sap had almost ceased to flow in the xylem of samples which had been subjected to sap tensions of about 4 MPa. However, because of considerable variability in the permeability of individual stems, it was not possible to determine the exact relationship between the permeability of the xylem and sap tension.

In Rhododendron cavitation occurs at sap tensions of from 1 to slightly over 3 MPa. The decrease in

stem permeability over the same range of sap tensions complements the occurrence of cavitation over a similar range of sap tensions. Moreover, the failure of cavitation to interrupt the supply of water to the leaves of whole plants (section 4.6) suggests that there is a considerable oversupply in the provision of xylem vessels in this species.

A high degree of redundancy of xylem conduits may occur in many plants (e.g. Mackay and Weatherly, 1973). Such redundancy might enable the supply of water to the leaves to continue almost unaffected by cavitation of a number of the conduits carrying sap. However extensive cavitation occurring at high sap tensions and affecting a high proportion of conducting conduits would eventually disrupt sap flow sufficiently to restrict the supply of water to the leaves. In Rhododendron this would be expected to occur if sap tensions exceeded about 2 MPa, the tension around which most cavitation occurs (section 3.5). Extensive cavitation, resulting in reduced stem permeability, may be the cause of slow equilibration of water potentials on Rhododendron shoots at sap tensions above 2 MPa (section 4.5). It has been suggested that extensive cavitation may 'isolate' some sap filled conduits by ringing them with cavitated, and therefore non-conducting, conduits (Milburn and Johnson, 1966). Further reductions in the water potential of the sap in these remaining conduits can only occur by movement of water in the high resistance pathways of the cell walls (Nobel, 1970). The water potentials of the sap in these conduits may therefore lag behind changes in leaf water potential, delaying cavitation of the sap in the remaining sap-filled conduits. A plateau-type cavitation profile, such as was found in Fraxinus, might result. However this is highly speculative.

Further work on the effect of high sap tension on xylem permeability is required in two areas. Firstly, experiments must be designed to preclude the possibility of cavitation occurring when water-stressed material is prepared for permeability experiments (Crafts et al., 1949). This might be done by measuring the permeability of the xylem in intact plants. Alternatively all manipulations may be carried out on rehydrated samples, in which sap tensions

are low, so that the possibility that artifactual cavitation might occur is reduced. This would require controlled rehydration of samples which have been subjected to cavitating sap tensions so that emboli in the xylem do not dissolve. Secondly, experiments should be conducted to determine the effect of cavitation on the water balance of whole plants. Oversupply of xylem conduits may serve to protect the water supply of the leaves from the effects of limited episodes of cavitation; whole plant studies are a way of determining if this is so.

From the permeability studies with Rhododendron it is reasonable to suggest that sap tensions must exceed those at the peak of the cavitation profile to affect seriously the flow of water in the xylem. How likely are plants in the field to experience sap tensions of this magnitude? According to Richter (1976) trees on mesic sites, such as those trees used in these studies, will seldom be subjected to sap tensions above 1.7 to 2 MPa. This is similar to the sap tensions at which the peaks of the cavitation profiles of leaves of the trees used in this study occur (section 3.5). Extensive cavitation may therefore occur occasionally in these species and require periodic replacement of the conducting xylem. However as sap may also be cavitated by winter freezing (Zimmermann and Brown, 1971) the requirement for annual replacement of the conducting xylem can not be attributed to high summer water deficits alone.

In contrast to trees, some herbs cavitate at relatively low sap tensions, e.g. 0.3 and 0.8 MPa in Lycopersicum and Ricinus respectively. But, from the examples given by Richter (1976), sap tensions in herbs are likely to be of the same order as those occurring in trees. Extensive cavitation in some herbs is therefore likely and may even be a daily occurrence (Milburn and McLaughlin, 1974). It will almost certainly be impossible for such herbs to grow new vessels to replace those which have cavitated. Sap flow in the cell walls, although possible (e.g. Scholander et al., 1955), will encounter very high resistances to flow (Nobel, 1970) and may not be sufficient to supply leaf water requirements without serious reductions in leaf water potential. Flow in the xylem can therefore be maintained only if cavitated xylem conduits are refilled. This may occur when sap pressure is raised above that of the

cavitation emboli, for instance by root pressure (Milburn and McLaughlin, 1974). Refilling of cavitated conduits in leaves or shoots supplied with water at or above atmospheric pressure has already been demonstrated (section 3.6, Milburn and Johnson, 1966; Milburn and McLaughlin, 1974). Cavitated conduits in trees are unlikely to be refilled as the height of trees often exceeds that to which root pressure can raise sap (Zimmermann and Brown, 1971).

The occurrence of cavitation in plants of very dry environments has not been investigated. Such plants may develop sap tensions of about 10 MPa (Richter, 1976) which would be expected to cause serious disruption of sap flow in all of the species used in this study. There is some evidence that cavitation occurs at higher sap tensions in plants (e.g. Eucalyptus (table 18), Fraxinus ornus (Milburn, pers. comm.)) from areas where droughts are frequent than in plants from areas with milder climates. If this is so study of cavitation in these plants, together with a study of their vascular anatomy, may be useful in isolating the factors which determine the sap tensions at which cavitation occurs in different plants. More generally study of cavitation in plants typical of particular environments will help our understanding of the importance of cavitation to the long term water relations of the plant. The ability of plants to 'adapt' or 'acclimatise' to particular environments by modifying the structure of the xylem as suggested by Carlquist (1975) (see section 1.10), or in other ways, may be a fruitful area for future research.

The experiments described in this thesis have shown that cavitation occurs in plants at sap tensions that may occasionally occur in their xylem. That cavitation does occur does not invalidate the cohesion theory of Dixon and Joly (1894) as cavitation is likely to be either infrequent or reversible, depending on the species. However this study has identified some of the factors limiting the cohesive ascent of sap in plants.

Table 18. Cavitating sap tensions found in other studies, some of which used methods other than acoustic detection to determine limiting sap tensions. Where possible, the sap tensions found to cause cavitation in the same species in this study are also included.

Species	Sap tensions causing cavitation (MPa)	Method used in determination	Reference
Acer saccharum	2.5	Dye injection	Greenidge (1955a)
Eucalyptus maculata	3.5 - 4.5	Acoustic detector and *in situ* hydrometer	Crombie and Milburn (1980)
Cotton (*Gossypium hirsutum*?)	1.0 - 2.5	Gas flow through stems frozen to preserve emboli	Byrne *et al.* (1977)
Lycopersicum esculentum	0.3 - 4.5+	Comparison of Ψ_l before and after xylem was cut	Cary *et al.* (1967)
" "	0.3 - 1.0	Acoustic detector and pressure chamber	Nonhebel (pers. comm.)
" "	0.2 - 0.4	" "	This study
Malus sylvestris	1.4 - 2.0	Acoustic detector, thermocouple psychrometer and pressure chamber	West and Gaff (1976)
Plantago major	1.0 - 1.2	Acoustic detector and pressure chamber	Milburn and McLaughlin (1974)
" "	1.4 - 1.9	" "	This study
Ricinus communis	0.2 - 0.9	" "	Milburn (1973b)
" "	0.4 - 0.8	" "	This study
Corn (*Zea mays*?)	2.5 - 3.85	Comparison of Ψ_l before and after xylem was cut	Cary *et al.* (1967)

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Appendix 1. Construction, calibration and operation of the thermocouple psychrometer sample chambers

Six psychrometer chambers suitable for use with the HR-33T Dewpoint Microvoltmeter (Wescor Inc., Logan, Utah) were built according to a design supplied by Dr. W.J. Davies of the Biological Sciences Department of the University of Lancaster.

The design of the chambers is illustrated in figure A.1.1. The chambers are in two parts, both milled from one inch (25.4 mm) brass rod.

A.1.1. Chamber construction

A 6 mm diameter by 1 mm deep circular recess in the lower half of the chamber serves as the sample chamber. A similar recess in the upper half contains the thermocouple for making water potential (Ψ) measurements. A 1 mm wide by 0.5 mm deep groove concentric to the central recess holds a rubber sealing ring which closes the chamber when forced against the smooth surface of the lower half of the chamber.

The chamber halves are aligned by two screws passing through holes drilled in the top and bottom chamber halves. Removal of all except the terminal part of the screw's thread allows the chamber halves to slide freely on them. The chambers were sealed by sliding the halves together along the screws. A consistent sealing pressure was obtained by a small compression spring mounted on a plate between the screws. The sealing pressure exerted by the spring could be adjusted by a nut on the treaded portion of one of the screws. Once adjusted, the sealing pressure was fixed by glueing the nut to the screw. The other end of the plate slid into a groove cut into the second screw. The spring is an addition to the design supplied by Dr. Davies.

Surfaces, particularly those inside the sample chamber, were milled as smooth as possible and hard chromed to minimise adsorption of water during equilibration and measurement of Ψ (Dixon, pers. comm.).

Figure A.1.1. Psychrometer sample chamber

1. Copper leads set in epoxy resin.
2. Copper binding posts.
3. Thermocouple.
4. Rubber O-ring.
5. Sample chamber.
6. Screws (thread stripped except at 10).
7. Chromed brass chamber body.
8. Compression spring.
9. Metal flange pivoted at 10.
10. Adjusting nut.

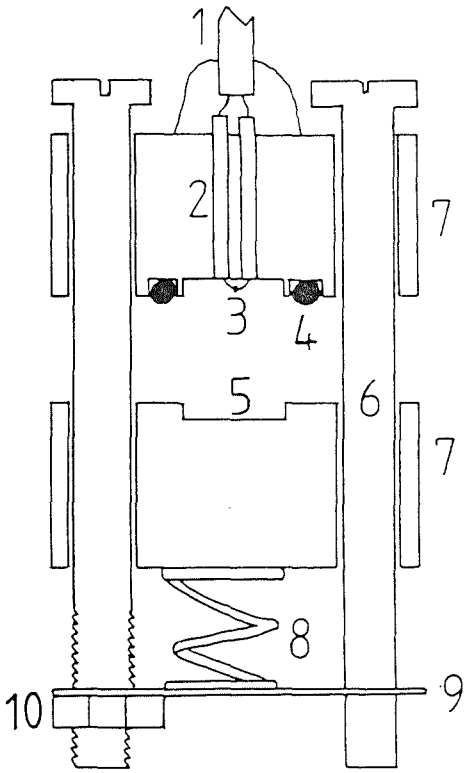
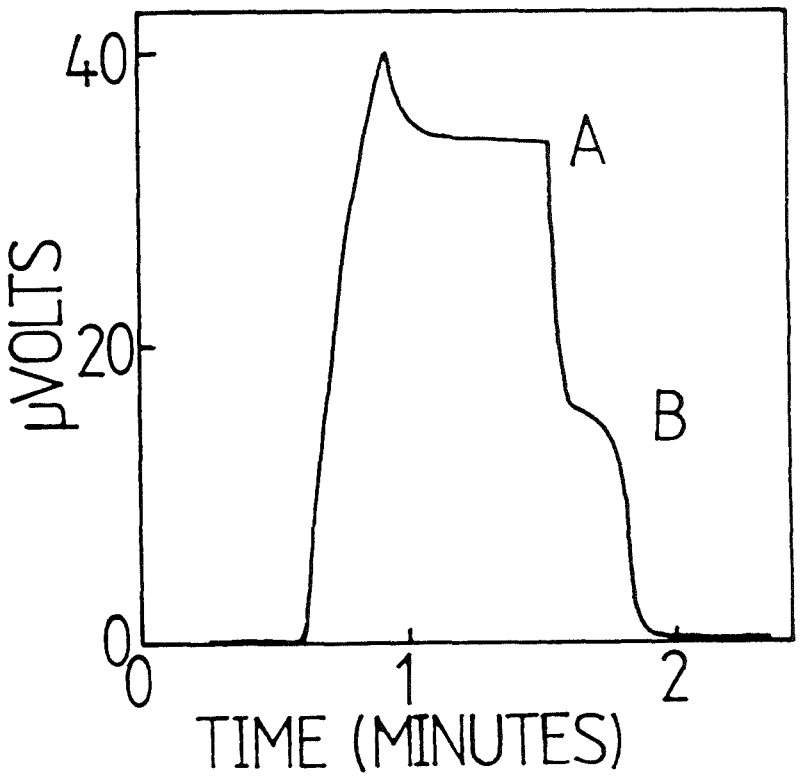


Figure A.1.2. Thermocouple output in A) dewpoint and B) psychrometric mode. Failure to establish a constant reading at A or a slow decline to zero from B indicates a dirty thermocouple.



Manufacture and mounting of thermocouple

Thermocouples were welded from .001 inch (.002 cm) chromel and constantan alloy wires (T1 alloy T1B/312 and Special Advance A873 respectively; supplied by British-Driver-Harris Co. Ltd., Stockport, England).

Thermocouples were tied eight at a time on a perspex jig (Campbell et al., 1968). After cutting, thermocouples were welded under glycerine by discharging a capacitor through them to an earthed carbon rod. Thermocouples were examined microscopically and irregularly shaped or corroded specimens discarded.

Thermocouples were mounted on their 1 mm copper lead wires by soldering them into pits punched into the ends of the wires.

Two 30-40 mm lengths of enamelled 1 mm copper wire were cut and the ends sanded smooth with fine sand-paper. A small conical hole was punched in the end of each wire using a sewing needle. The holes were filled with solder by touching a hot soldering iron to the opposite end of the wire and melting flux cored solder into the hole at the other end. The end was polished again to remove excess solder. The wires were then mounted in a wooden jig at the same spacing as in the psychrometer chambers. The solder was remelted by again touching a soldering iron to the opposite end of the wires and one end of the thermocouple (trimmed to about 7 mm in length) embedded in the solder filled pit of each wire. The mounted thermocouple was checked for electrical continuity and if necessary remounted.

The lead wires with the mounted thermocouple were removed from the jig and slipped through the mounting holes in the upper half of the psychrometer chamber. After positioning with the surfaces of the lead wires flush with the inner surface of the psychrometer chamber and the thermocouple not touching the chamber, the thermocouple was checked for continuity and shorts to the chamber body. If satisfactory, the lead wires were secured by a drop of cyanoacrylate resin applied to the outside edge of the mounting holes. The low viscosity resin penetrated to the inner edge of the holes before solidifying and fixing the wires firmly in place.

Mounting of the thermocouples in solder as described was preferred to mounting in slits cut into the lead wires, as in the Wescor devices, as it was easier to clean the thermocouple chambers if mounted in this way. Spurious EMF's generated by the extra junctions with the solder were not found to be a problem in operating the chambers.

Cleanliness of the psychrometers was critical for their successful operation.

The psychrometers were washed in 5% Lipsol detergent solution (LIP (Equipment and Services) Ltd., Shipley, England) and rinsed twice under running distilled water. After rinsing, a drop of water was deliberately left in the thermocouple chamber. This drop was blown off the thermocouple by using a commercial freezing aerosol (Fison's Freezing Aerosol Spray, Fison Scientific Apparatus, Loughborough, England), carrying the last traces of contaminant with it. Cylinder compressed air was used initially for this operation but was found to contaminate the thermocouple, possibly through deposition of oils from the regulators or tubing used.

The rubber sealing ring was washed separately by the same procedure and remounted in the chamber after both had been dried.

Cooling co-efficients (π_v) of the thermocouples (i.e. the thermocouple EMF resulting when the temperature of the junction is altered by passing a current through the junction) were between 65 and 80 μV when read from the microvoltmeter as directed by the Wescor manual. This value remained constant over the two years over which the psychrometers were used. π_v of these thermocouples is comparable to the 50-60 μV of similar junctions supplied by Wescor.

A.1.2. Calibration and Operation

The chambers were calibrated using NaCl solutions soaked onto 6 mm discs of Whatman number one filter paper. A range of calibration solutions with water potentials between 0.0025 MPa and 4.5 MPa were obtained using the tables

of Lang (1967). Calibration solutions between zero and the lowest of those quoted by Lang (1967) were estimated from a straight line interpolation of Ψ against salt concentration through these known points. At low concentrations the relation between salt concentration and Ψ is very nearly linear, and no appreciable error is incurred in using the straight line approximation.

The Wescor WR-33T Dewpoint Microvoltmeter was operated in accordance with the procedure laid out in the operating manual with one exception, the setting of the initial cooling cycle. To avoid 'jumps' in calibration which might occur when a standard, short cooling time for measuring high Ψ values was changed to a longer interval for measuring samples of low Ψ a two stage procedure was adopted. A first cooling cycle was run and the approximate dewpoint noted. The condensed droplet was then evaporated from the thermocouple by a brief heating cycle and the chambers left for several minutes to re-equilibrate. A second cooling operation followed, this time to 5 μV beyond the approximate dewpoint of the first cycle. The dewpoint reading on this second cycle was noted and the calibration for the chamber consulted to obtain the sample water potential.

Microvolt measurements for a single sample were replatable to ± 0.1 μV. Variability was slightly greater at higher (less negative) Ψ and accuracy fell rapidly at Ψ higher than -0.1 MPa.

Psychrometer sensitivity in the dewpoint mode was between 6.5 and 7 μV MPa^{-1} for all six sample chambers. Psychrometric sensitivity (produced by steady-state evaporation from the thermocouple junction) was approximately half this. Thermocouple EMF during a dewpoint and subsequent psychrometric measurement of Ψ_1 above a 0.8 Molal NaCl solution is shown in figure A.1.2.

The two-part construction of the psychrometer chambers resulted in a slow rate of thermal equilibration of the psychrometers and left them sensitive to gradients in temperature. Consequently control of temperature was essential to obtain accurate measurements of water potential. The psychrometers were equilibrated in metal centrifuge tubes extended by plastic cylinders submerged in a water bath maintained at 293±0.5 K by a Grant Flow

Cooler (FH15, Grant Instruments Ltd., Cambridge, UK). The centrifuge tubes were plugged with cotton-wool during equilibration to further reduce thermal gradients.

Earth loops between psychrometers in the water bath and the dewpoint microvoltmeter caused serious errors unless dissipated by connecting the water bath and microvoltmeter to a common ground.

Appendix II. The acoustic detector

The acoustic detector is a device for amplifying the vibrations produced by the cavitation of xylem sap. The detector used in these experiments had additional circuits for the reduction of extraneous noises and to enable automatic recording of cavitation.

With the exception of the power supply and the final stages of the anti-coincidence circuit all circuits were duplicated, to give two identical detectors working in parallel. Although both channels were sometimes used to detect cavitation in different samples one detector was usually used as a 'control' to detect extraneous noise in the laboratory, as well as electrical noise. The output of the 'control' channel was subtracted from that of the 'active' channel to give an output free of false events.

In the circuit diagrams below only one of each pair of circuits is shown.

Resistors used were $^{1}/_{3}$ watt, 5%, of the carbon film.

Capacitors < 10 F were polyester, > 10 F were aluminium electrolytic with the exception of the tuning capacitors of the monostable multivibrator circuit (circuit 4) which were tantalum.

All resistances are given in ohms.

Circuit 1. Power supply.

Outputs ± 12 V., + 5 V.

C_1, = 470 µF.

C_2 = "

C_3 = "

C_4 = "

C_5 = "

D1 = 1N4148

D2 = BZY83C 5.6 V

L1 = LM78L12

L2 = LM79L12

R1 = 150 Ω

R2 = 220 Ω

R3 = 100 Ω

T1 = TIP3055

T2 = BFY51

W = WO4 400 V, 1 Amp Bridge Rectifier

Circuit 2. Head amp. (gain x2000)

C1 = 47 nF

C2 = 47 nF

C3 = 2 α 47 nF

C4 = 100 nF

IC1 = LM324

R1 = 820 K Ω

R2 = 1.8 K

R3 = 270 K

R4 = 68 K

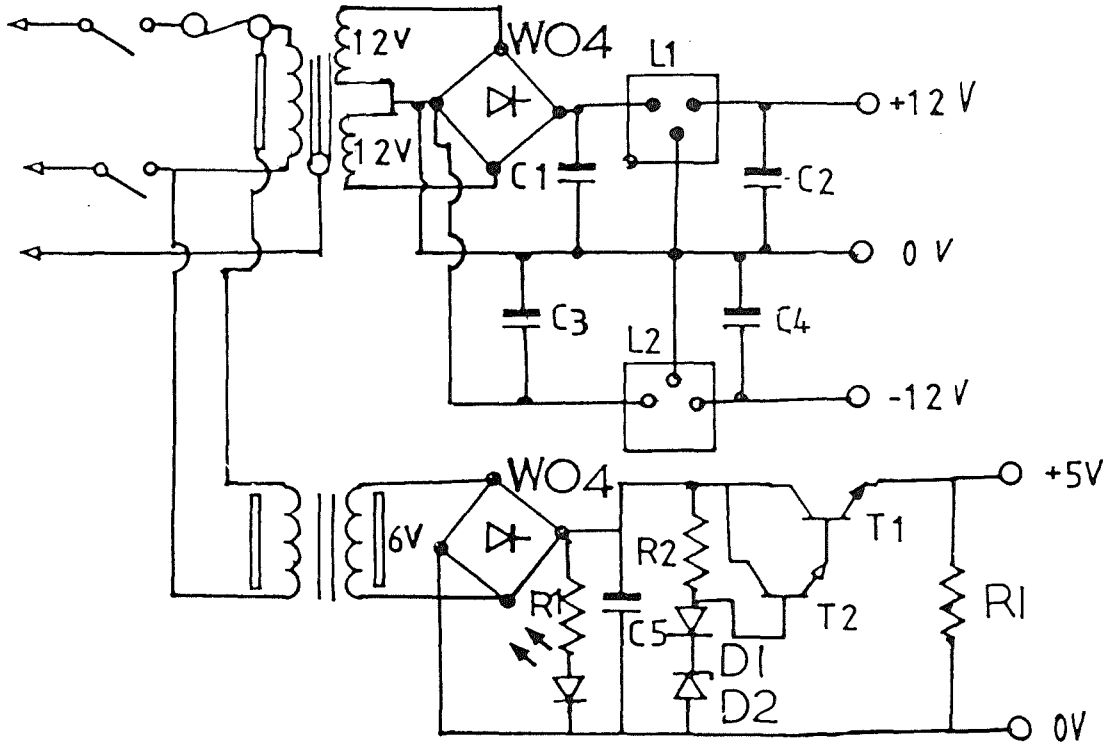
R5 = 68 K

R6 = 820 K

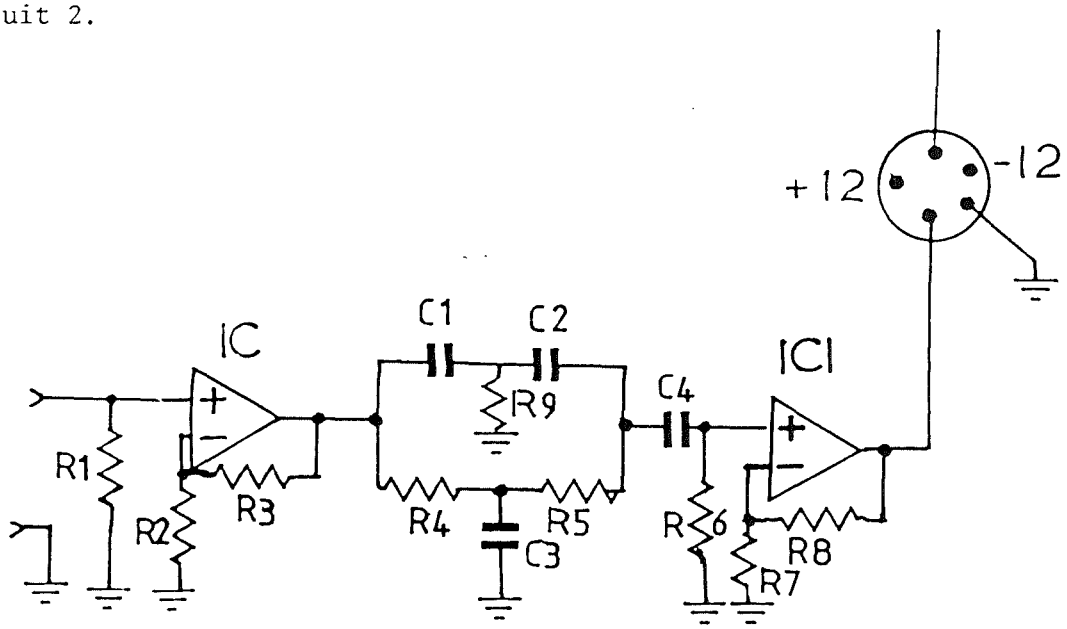
R7 = 8.2 K

R8 = 270 K

Circuit 1 .



Circuit 2.



Circuit 3. Main amplifier (gain x8)

This circuit includes 1) a high pass filter (to remove low frequency noise from the electrical mains, and 2) an envelope detector with adjustable response time (to prevent each of the several voltage peaks originating from a single cavitation event being counted as a separate event) and 3) variable threshold discriminator.

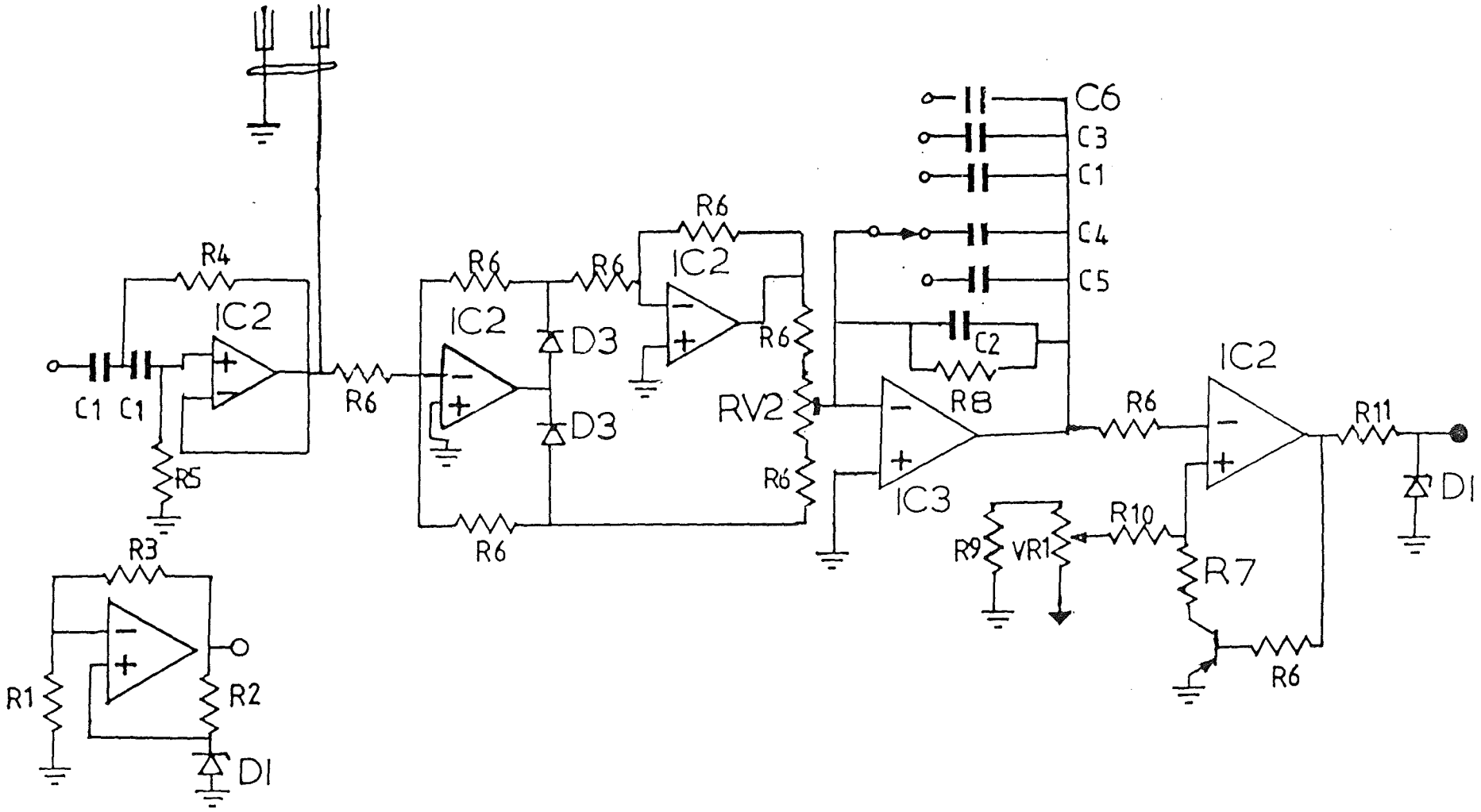
C1 = 68 nF
C2 = 4 nF
C3 = 10 nF
C4 = 100 nF
C5 = 220 nF
C6 = 470 nF

D1 = 5.6 V
D2 = 1N4148
D3 = IN4148

R1 = 39 K Ω
R2 = 330 K Ω
R3 = 10 K
R4 = 10 K
R5 = 22 K
R6 = 22 K
(R11) R7 = 1.5 K
R8 = 220 K
R9 = A.O.T. (330 Ω channel A, 680 Ω channel B)
VR1 = 10 K (Discriminator threshold)
RV1 = 10 K (Balance potentiometer)

IC2a = LM324
IC3 = LM741

Circuit 3.


C6
C3
C1
C4
C5
C2
R6
IC2
R4
IC2
R6
R6
IC2
D3
D3
R6
C1 C1
R5
R6
RV2
R8
IC3
R6
IC2
R11
D1
R3
R9
VR1
R10
R7
R6
R1
R2
D1

Circuit 4. Anti-coincidence circuit. Dual monostable multivibrators are used to shape pulses passed by the threshold discriminators of the main amplifier (circuit 3). The same circuits then act with a nand gate to eliminate signals appearing within 100 ms of each other on both channels.

IC4 = LM74123 (dual monostable multivibrators)

IC5 = LM74123 " " "

IC6 = LM74123 " " "

IC7 = LM7400 (quad nand gate)

Time constants:

IC4 (a) 10 μF, 22 K Ω, 50 K Ω preset

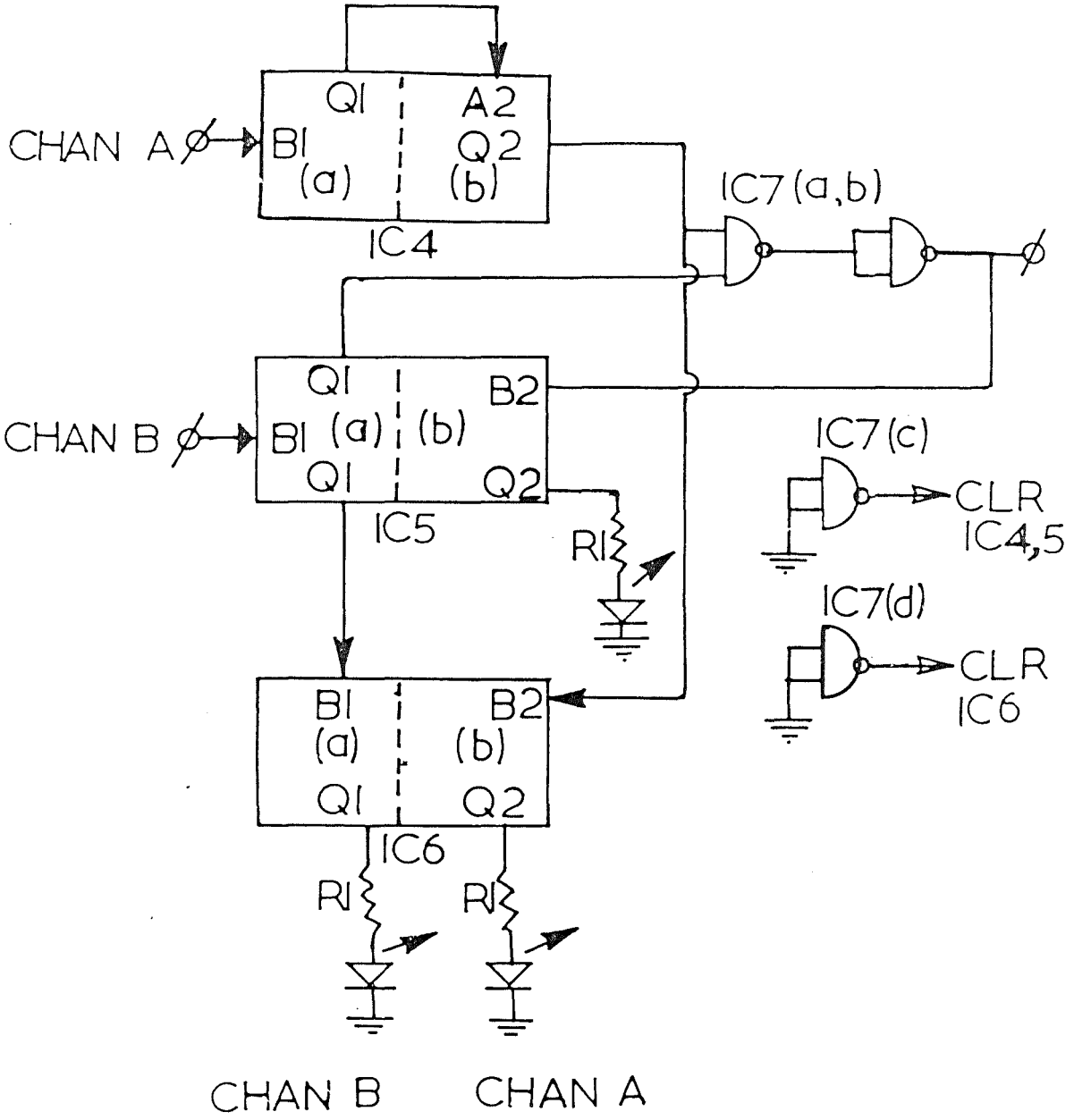
IC4 (b) 1 μF, 2.2 K Ω, 10 K Ω preset

IC5 (a) 10 μF, 22 K Ω, 50 K Ω preset

IC5 (b) 10 μF, 47 K Ω

IC6 (a,b) 10 μF, 47 K Ω

Circuit 4.


Q1
A2
CHAN A
B1
Q2
(a)
(b)
IC4
IC7 (a,b)
Q1
B2
CHAN B
B1
(a)
(b)
Q1
Q2
IC5
IC7 (c)
CLR
IC4,5
R1
IC7(d)
CLR
IC6
B1
B2
(a)
(b)
Q1
Q2
IC6
R1
R1
CHAN B
CHAN A

Errata

Throughout the text butan-1-ol has been referred to as N-butanol.

Throughout the text for 'Weatherly' read 'Weatherley'

Missing reference: Hipkins, M.F. (1978) Kinetic analysis of the chlorophyll fluorescence inductions from chloroplasts blacked with 3-(3,4-dichlophenyl)-1,1-dimethylurea. Biochim. Biophys. Acta 502: 514-523